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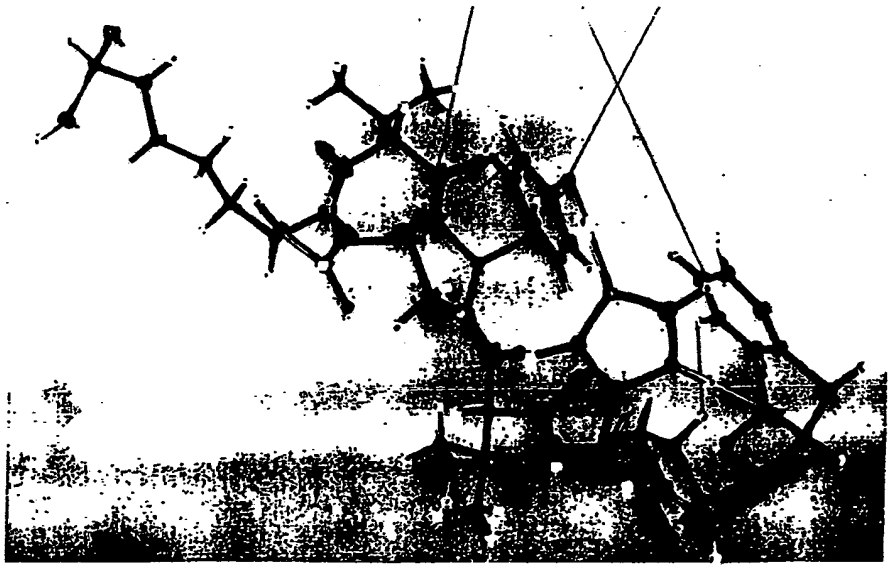
(54) Title: METHODS FOR MODELLING TERTIARY STRUCTURES OF BIOLOGICALLY ACTIVE LIGANDS INCLUDING AGONISTS AND ANTAGONISTS THERETO AND NOVEL SYNTHETIC ANTAGONISTS BASED ON ANGIOTENSIN



(57) Abstract

Disclosed are methods f r modelling the three-dimensional structure (tertiary structure) of a ligand having ne or more active sites empl ying a charge-transfer interaction. Also discl sed is a model f r Angiotensin II derived from such method as well as novel synthetic antagonists based on angiotensin.

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METHODS FOR MODELLING TERTIARY STRUCTURES OF BIOLOGICALLY ACTIVE LIGANDS INCLUDING AGONISTS AND ANTAGONISTS THERETO AND NOVEL SYNTHETIC ANTAGONISTS BASED ON ANGIOTENSIN

BACKGROUND OF THE INVENTION

1. Field of the Invention.

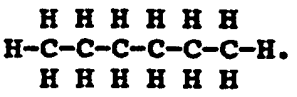
The present invention is directed to methods for modelling tertiary (three-dimensional) structures of biologically active ligands, to methods for designing and synthesizing agonists and antagonists to the ligands based on the three-dimensional models generated for such ligands, and to the model itself generated for Angiotensin II from the methods of this invention.

2. State of the Art.

In the field of chemistry, compounds can be defined in several ways. For example, a compound can be defined by its empirical formula, e.g., in the case of n-hexane the empirical formula would simply be C_6H_{14}. For simple molecules such as water, methane, carbon dioxide, etc., the empirical formula can provide useful information.

However, as the complexity of the molecule increases, the empirical formula must be complemented by structural information concerning the covalent bonding of the individual atoms vis-à-vis each other in order to derive meaningful information concerning the molecule. Such information is gen rally depicted as a

the covalent bonds between the respectiv atoms. Such primary structures are well known pictorial representations of the compound of interest. These representations are usually defined as the structural formula of the compound which, for example, in the case of say n-hexane would be represented as:



However, even with the molecule's structural formula, valuable information is still missing regarding the position in three-dimensional space of the individual atoms relative to each other. Such three-dimensional structures or conformations for a molecule are determined in part by non-covalent interactions, e.g., electrostatic and non-electrostatic interactions such as ionic interactions, hydrogen bonding, Van der Waal forces, etc., between different atoms of the molecule.

Three-dimensional information, i.e., the ligand's conformation, is extremely valuable for naturally occurring biologically active ligands. In particular, such biologically active ligands generally have one or more active sites on or within the molecular structure of the ligand. Such active sites can involve a charge-transfer interaction (as later defined). When such a ligand is bound to its complementary receptor molecule, the active site activates the receptor molecule thereby affecting the biological activity of the receptor molecule. Thus, activation of the active site, whether by a charge-transfer interaction mechanism or by some other mechanism, is generally a necessary step in affecting the biological activity f the receptor. Further in

this regard, if it were possible to create an accurate three-dimensional model of the naturally occurring biologically active ligand [including its active site(s)] as found in vivo, then such models could be used to create mimetics, e.g., agonists and antagonists, of such ligands. For example, if it is desirable to suppress the biological activity of the receptor in vivo, then an accurate three-dimensional model of the receptor's naturally occurring complementary ligand including its active site(s), would greatly facilitate the preparation of antagonists to this receptor. Likewise, an accurate three-dimensional model of the ligand of interest would also facilitate the design and synthesis of agonists when it is desirable to increase or to stimulate the biological activity of the receptor in vivo.

While three-dimensional models have heretofore been proposed for molecules including ligands, such three-dimensional representations have suffered from one or more serious drawbacks, particularly as they relate to biologically active ligands having active site(s) which employ a charge-transfer interaction. In particular, such prior art methods have failed to provide a simple means to identify the active site(s) of such ligands. Accordingly, in such cases, the creation of a three-dimensional model of such a ligand including its active site was generally conducted by extremely laborious procedures such as structure-activity relationships, theoretical considerations, etc. However, because such procedures are unable to identify a charge-transfer interaction at the active site of these ligands, it has

not been possible to model mimetics of such ligands to a meaningful conformation.

Additionally, other art recognized methods of modelling the tertiary structure of a compound in three-dimensional space, such as x-ray crystallography, have the drawback that with biologically active ligands, the steps required to prepare the ligand for analysis can change the ligand's tertiary structure and accordingly, the structure as determined by this analysis may not conform to the structure found in vivo. Moreover, not all biologically active ligands are amenable to such analysis.

In view of the above, it is an object of this invention to develop a process which would model the three-dimensional spatial (tertiary) structure of a biologically active ligand having one or more active sites employing a charge-transfer interaction. It is a further object of this invention that this modelling identify the chemical groups at the site(s) of charge-transfer interactions. It is still a further object of this invention to create models of such ligands closely resembling the structure of the ligand found in vivo. It is still another object of this invention to design mimetics to such ligands by reference to the model generated for the ligand. These and other objects are achieved by the present invention as evidenced by the attached summary of the invention, detailed description of the invention, examples and claims.

SUMMARY OF THE INVENTION

The above objectives are achieved by the methods of the present invention. In particular, by using these methods, one is now able to model biologically active ligands having one or more active site(s) which employ charge-transfer interactions. The methods of the present invention involve identification of a charge-transfer interaction using fluorescent methods, identification of the groups involved in the charge-transfer interaction by structure-activity studies, and application of NMR methods to resolve remaining aspects of the conformation surrounding the charge-transfer interaction. Moreover, the model or conformation so obtained is used in a method to design mimetics, i.e., agonist and antagonists, of such ligands. Accordingly, in one of its method aspects, the present invention is directed to a method for creating a three-dimensional spatial model for a biologically active ligand having one or more active sites based on a charge-transfer interaction and further having a known structural formula wherein the three-dimensional spatial assignments for each of the atoms of the ligand in the model are assigned from the steps comprising:

a) determining the presence of charge-transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment;

b) determining the chemical groups involved in said charge-transfer interaction(s); and

c) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to the active sit (s) from nucl ar magnetic resonanc spectroscopy

employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000.

Another method aspect of the present invention is directed to a method of modelling antagonists to a biologically active receptor based on the model generated for a biologically active ligand complementary to said receptor wherein said ligand has one or more active sites based on a charge-transfer interaction and further has a known structural formula which method comprises the steps of:

a) creating a three-dimensional spatial model for said ligand by

i) determining the presence of charge-transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment;

ii) determining the chemical groups involved in said charge-transfer interaction(s); and

iii) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to the active site(s) from nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000; and

b) identifying a compound having a three-dimensional structure sufficiently similar to said ligand so as t be complementary to said r ceptor and

wherein at least one of the charge-transfer interactions in said compound has been compromised.

Still another method aspect of the present invention is directed to a method of modelling agonists to a biologically active receptor based on the model generated for a biologically active ligand complementary to said receptor wherein said ligand has one or more active sites based on a charge-transfer interaction and further has a known structural formula which comprises the steps of:

a) creating a three-dimensional spatial model for said ligand by

i) determining the presence of charge-transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment;

ii) determining the chemical groups involved in said charge-transfer interaction(s); and

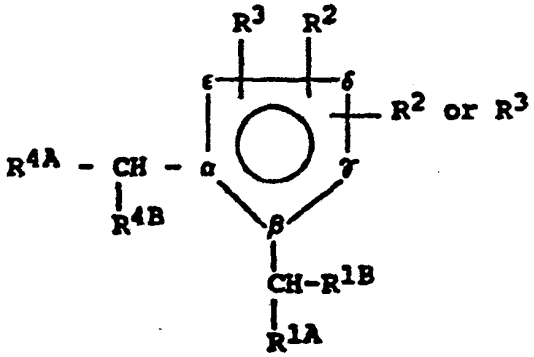
iii) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to the active site(s) from nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000; and

b) identifying a compound having a three-dimensional structure sufficiently similar to said ligand so as to be complementary to said receptor and wherein the charge-transfer interaction(s) in said compound has (have) not been compromised.

Yet another method aspect of the present invention is directed to a method for determining the presence of charge-transfer interaction(s) in the tertiary structure of a biologically active ligand complementary to a biologically active receptor which comprises conducting fluorescence analysis of said ligand in a fluorescence compatible environment.

In a preferred embodiment, the above described methods are particularly suitable for modelling a three-dimensional spatial structure of Angiotensin II. FIGURE 6 of this application illustrates a stereo photograph of a molecular model (three-dimensional model) for Angiotensin-II. FIGURE 8A of this application illustrates a stereo photograph of a molecular model (three-dimensional) for receptor-bound Angiotensin II. Accordingly, another aspect of this invention is directed to the model of Angiotensin-II illustrated in FIGURE 6 as well as the model of receptor-bound Angiotensin II illustrated in FIGURE 8A.

A product aspect of the present invention is directed to a compound of the formula:

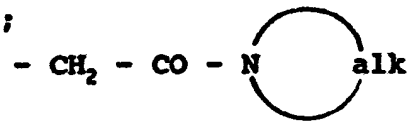


wherein $\alpha,\beta,\gamma,\delta$ and ϵ are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom, and (b) attachment of R groups is to C or N, and preferably further with the provisos that (c) at least one ring N atom remains unsubstituted, and (d) the pKa of the ring is ≤ 7 when all attendant groups have been taken into account;

R^{1A}, which mimics the structure in angiotensin of - CH - CO - N - CH - CO -

includes the following:

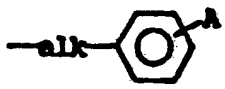
-alk; -O-alk; -alk-O-alk; $-CH_2-CO-NH_2$; $-CH_2-CO-NH-alk$; $-CH_2-CO-N(alk)_2$;



$-CH_2-CO-AA-NH_2$; or $CH_2-CO-AA-Phe$,

wherein AA is an amino acid preferably proline, azetidine-carboxylic acid, pipecolic acid, nipecotic acid, glycine, alanine, sarcosine, or N-methyl-alanine;

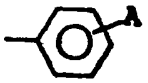
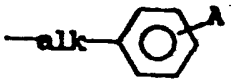
R^{1B}, which optionally provides a spacer arm terminating in a mimic of the C-terminal carboxylate group of angiotensin II, includes the following:

-H; -alk-A;  ; or  ;

preferably with the proviso that when R^{1B} is H, then: (a) if the ring is imidazole α and/or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2;

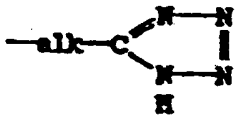
R^2, which provides steric and/or electronic properties and/ r a spac r arm terminating in an acid group, includes the following: -H, -halide; -alk;

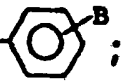
-O-alk; $-NO_2$; $-CF_3$; -CN; -alk-A; -A;


; or


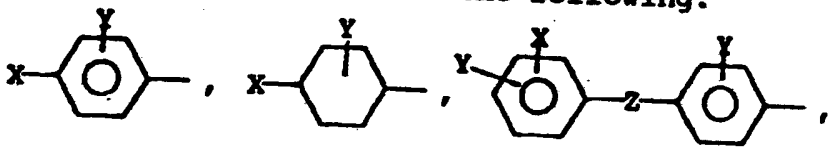
R^3, which provides steric and/or electronic properties and/or a mimetic of the tyrosine hydroxyl group of angiotensin II in its "charge relay" conformation, or a spacer arm terminating in a mimic of the N-terminus of N-terminal dipeptide of angiotensin-II, includes the following;

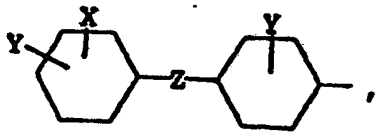
-H; -alk; -aryl; -alk-OH; -alk-halide; $-CH_2$-O-alk; $-CH_2-CN$; $-CH_2-CO_2H$; $-CH_2CO_2$-alk; -NH-CO-alk; -CO-NH-alk; -alk-B; -CH(OH)-alk-B; -alk-Asp-Arg-NH_2; -CH(OH)-alk-Asp-Arg-NH_2;

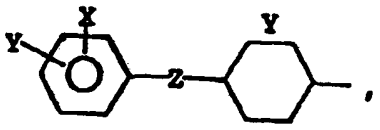

;

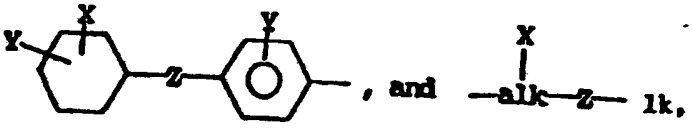
; or

;

R^{4A}, which provides a spacer arm, the relative rigidity of which is an aspect of the design, terminating in an acid group which mimics the tyrosine hydroxy groups of angiotensin II in its "receptor bound" conformation includes the following:





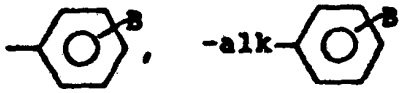



, and

1k,

where Z is a bond, -NHCO-, -O-, $-OCH_2-$, or $-CH_2-$;
X is $-CO_2H$, -alk-CO_2H, $-PO_3H$, -alk-PO_3H, $-PO_4H_2$, -alk-PO_4H_2, -SH, -alk-SH, $-SO_3H$, -alk-SO_3H, $-SO_4H_2$, -alk-SO_4H_2, $F_3C-CO-NH-$, F_3C-SO_2-NH-,

or yet another acid group
or a pharmaceutically acceptable salt thereof; and
Y is -H, -halide, $-NO_2$, -O-alk, -alk, $-CF_3$, or -CN; and
R^{4B}, which optionally provides a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin, includes the following:
-H, -alk-B, -alk-Asp-Arg-NH_2, alk-O-alk-B, alk-O-alk-Asp-Arg-NH_2,

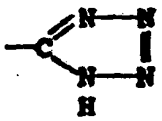


preferably with the proviso that when R^{4B} is H, then: (a) if the ring is imidazole α and/or γ is other N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2;

alk = an alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having 3-6 carbon atoms,an alkenyl group having 2-10 carbon atoms, or an alkynyl group having 2-10 carbon atoms;

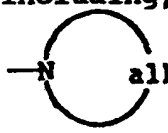
halide = -F, -Cl, -Br, or -I;

A = an acid group or its pharmaceutical salt and includes but is not limited to $-CO_2H$, $-CO_2R^*$, $-CO_2$alk, $-SO_3H$, $-SO_4H_2$, $-PO_3H$, $-PO_4H_2$, $F_3CCONH-$, F_3CSO_2NH-, -alk-SH, or



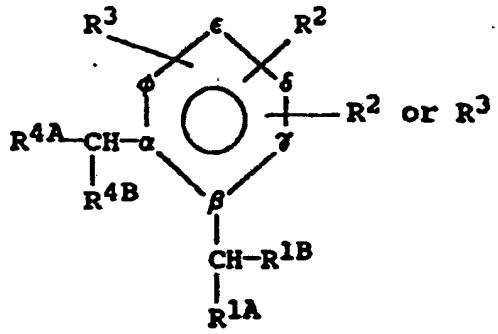
wherein R^+ is a lipophilic st r prodrug form such as $-CH_2CO_2C(CH_3)_3$ and the like;

B = a basic group or its pharmaceutical salt including, but not limited to $-NH_2$, -NHalk, $-N(alk)_2$,



In a particularly preferred product aspect of the present invention, the above five-membered ring is imidazole.

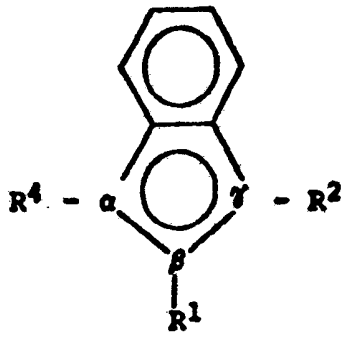
Another product aspect of the present invention is directed to a compound of the formula:



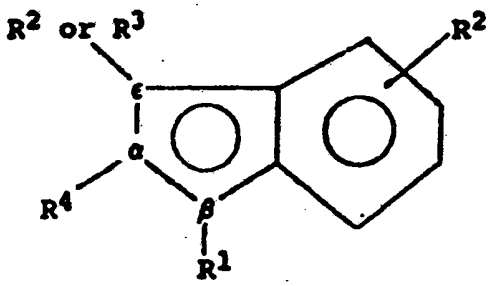
wherein α, β, γ, δ, ϵ, and ϕ are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom, and (b) attachment of R groups is to C or N, and preferably further with the provisos that (c) at least one ring N atom remains unsubstituted, and (d) the pKa of the ring is ≤ 7 when all attendant groups have been taken into account;

and wherein R^{1A}, R^{1B}, R^2, R^3, R^{4A}, Z, X, and Y, R^{4B}, alk, halide, A, and B are as defined previously.

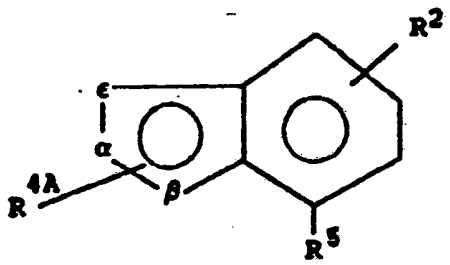
Another product aspect of the present invention is directed to a compound of the formula:



Configuration I



Configuration II



Configuration III

wherein α, β, and γ are C or N, with the proviso that only one N atom is substituted. R^1 is $-CH(R^{1A})(R^{1B})$ and R^4 is $-CH(R^{4A})(R^{4B})$. $R^5 = R^1$. Substituents R^{1A}, R^{1B}, R^2, R^3, R^{4A} and R^{4B} are as defined above, except that for these compounds when R^{1B} is H then (a) R^{1A} comprises a group containing an amide, or (b) R^1 is on an N or (c) R^4 is on a C.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates a molecular model (three-dimensional spatial mod l) for [Sar^1]Angiot nsin II dev l ped by the methods of th present invention.

FIGURE 2A illustrates a two-dimensional representation of the Angiotensin II antagonist, Sarmesin, i.e., [$Sar^1Tyr(Me^4)$]Angiotensin II.

FIGURE 2B illustrates a two-dimensional representation of the Angiotensin II.

FIGURE 2C illustrates a two-dimensional representation of the Angiotensin II antagonist, Sarilesin, i.e., [Sar^1Ile^8]Angiotensin II.

FIGURE 3A illustrates a two-dimensional representation of one example of an N-benzyl-imidazole compound and FIGURE 3B illustrates a two-dimensional representation of one example of an N-benzamidobenzyl-imidazole, both compounds are in a class of compounds which are Angiotensin II antagonists.

FIGURES 4A illustrates a molecular model (e.g., a three-dimensional spatial model) for Angiotensin II developed by the methods of the present invention whereas FIGURE 4B illustrates a charge distribution map for Angiotensin II obtained obtained by overlaying the relative charges found in Angiotensin II onto the model illustrated in FIGURE 4A.

FIGURE 5A illstrates a two-dimensional structural formula of the imidazole portion of Angiotensin II whereas FIGURE 5B provides an overlay of the common portions of the compounds illustrated in FIGURES 3A and 3B and depicted by solid lines onto the imidazole portion of Angiotensin II illustrated in FIGURE 5A and depicted by dashed lines.

FIGURE 6 is a stereo photograph of a model of Angiotensin II produced by the methods of this invention.

FIGURE 7A is a stereo photograph of the receptor bound form of Angiotensin II. FIGURE 7B is a stereo photograph of the overlay of the compound illustrated in FIGURE 7C over the receptor bound form of Angiotensin II set forth in FIGURE 7A.

In the molecular models 2cm = 1 Å. One skilled in the art will recognize that when the models of Figures 6 and 7 are constructed from Minit Molecular Models, Chocranes, Oxford, U.K., the color models will provide even easier perception of the configurations than the black and white stereo photographs herein provide. (Additionally, it will be recognized by one skilled in the art that in FIGURES 1, 4, 6 and 7, an apparent error appears in that the valine and aspartic amino acids are inadvertently depicted in the D rather than their correct L configuration.)

DETAILED DESCRIPTION OF THE INVENTION

Although investigations on the conformation of naturally occurring biologically active ligands such as Angiotensin-II have heretofore been carried out, such investigations generally did not take into account the presence of a charge-transfer interaction in the ligand which is required for receptor activation, and therefore it has not heretofore been possible to readily model ligands as well as mimetics of such ligands to a meaningful conformation. However, by the methods of the present invention which do take into account charge-transfer interactions, it is now possible to model biologically active ligands having

active site(s) which employ charge-transfer interactions to a meaningful conformation. Moreover, it is also possible to use the methods of the present invention to model mimetics of such ligands. However, prior to discussing this invention in detail, the following terms will first be defined.

"Charge-transfer interaction" -- is an electrostatic interaction involving a phenol residue in which an anionic charge is transferred from a charged group to an uncharged group. In one embodiment, the phenol residue is initially uncharged, i.e., phenol, and as a result of the charge-transfer interaction, this residue accepts an anionic charge from another charged group; thus in this embodiment the phenol residue becomes a phenolate residue. In another embodiment, the phenol residue is initially charged, i.e., phenolate, and as a result of the charge-transfer interaction, this residue transfers its anionic charge to an originally uncharged group; thus in this process, the phenolate residue becomes a phenol residue.

Any phenol residue found in a biologically active ligand can be employed in the charge-transfer interaction. Suitable phenol residues include those found in the amino acid tyrosine and derivatives thereof, in steroids having a phenol group such as estradiol [estra-1,3,5(10)-triene-3,17,diol] and derivatives thereof, in catecholamines such as norepinephrine and derivatives thereof, in naphthol containing ligands and the like. The above list is not meant to be an exhaustive representation of naturally occurring components employing phenol residues but rather is presented for the purpose of illustrating

that such phenol residues can be found in many different biologically active ligands.

"Active sites based on charge-transfer interactions" -- refers to activation site(s) in a biologically active ligand (for activating a biologically active receptor) which is (are) based on an electrostatic interaction involving a phenol residue in which an anionic charge is transferred from a charged residue to an uncharged residue. Accordingly, in such interactions at least one of the residues is either a phenol residue or a phenolate residue. In such ligands, activation of the receptor by the ligand cannot occur without the charge-transfer interaction. Charge-transfer interactions have heretofore been suggested for ligands such as Angiotensin II. See, for instance, Moore et al., Bioscience Reports, 5, pp. 407-416 (1985), which proposed that tranfer of a negative charge from the C-terminal carboxylate residue through the imidazole residue of the histidine amino acid to the tyrosine side chain results in the formation of a phenolate species which upon interaction with the receptor activates the Angiotensin II receptor. Such charge-transfer interactions allow the ligand to modify its electrostatic character into a form which allows activation of the receptor.

The charge-transfer interaction need not be an electrostatic interaction confined solely to the ligand but also could involve a transfer of charge from either a residue on the ligand to a residue on the receptor, or from a residue on the receptor to a residue on the ligand, said transfer being a necessary pr condition t activation of the receptor by the

ligand. For example, th formation of the tyrosinate species on the ligand can be the result of the transfer of an anionic charge from an anionic residue on the receptor. Upon formation of the tyrosinate species, the ligand is then capable of activating the receptor.

The methods of the present invention employ techniques which permit detection of charge-transfer interactions in biologically active ligands or in biologically active ligand/biologically active receptor complexes. These techniques employ a fluorescence analysis discussed below in a fluorescence compatible environment.

"Ligand" -- any organic compound for which a receptor naturally exists or can be prepared.

"Biologically active ligand" -- a molecule which binds to a biologically active receptor molecule and which directly or indirectly affects the activity of the receptor molecule. Binding of such ligands to the receptor (acceptor) molecule is accordingly a necessary precondition for initiating, terminating, altering or preventing the biological activity in the receptor molecule. Any ligand which affects the biological activity of the receptor molecule is said to be a biologically active ligand. The biologically active ligand can be a substrate, an agonist, an antagonist, an activator, an inhibitor, etc. When a ligand is able to bind to a specific receptor, the ligand and receptor pair are said to be complementary. Examples of biologically active ligands are well documented in the art. Examples of important bi logically active ligands includ , for example,

oxytocin (wherein the presently known complementary receptors are oxytocin receptor and oxytocin-neurophysin), vasopressin (wherein the presently known complementary receptors are the V_1 receptor, the V_2 receptor, and vasopressin neurophysin), Angiotensin II (where the presently known complementary receptor is known as the Angiotensin II receptor), and the like.

The biologically active ligand can be peptidic or non-peptidic in nature. Such ligands can be indigenous to the organism where the biologically active receptor is found. When the ligand is one which is naturally occurring in that organism, then that ligand is referred to as a naturally occurring biologically active ligand. On the other hand, the biologically active ligands can be synthetic molecules which are complementary to the biologically active receptor and which affect the biological activity of the receptor. Thus any molecule which is complementary to a biologically active receptor and which affects the biological activity of the receptor, is a biologically active ligand.

When binding of the biologically active ligand to the biologically active receptor and the activation of the active site results in an alteration of the biological activity of the receptor, e.g., initiates, increases, decreases or terminates the biological activity of the receptor, the ligand is said to directly affect the activity of the receptor. On the other hand, a biologically active ligand indirectly affects the activity of the biologically active rec ptor when the binding of the ligand to the rec ptor results in an inability t activate the rec ptor

(because the ligand possess a compromised charge-transfer interaction--as in the case of a antagonist).

Activation of the active site of the naturally occurring biologically active ligand/receptor complex is generally accomplished by some sort of chemical interaction within the ligand or between the ligand and the receptor. As noted above, when the chemical interaction involves the transfer of charge from one residue to another wherein one of the residues is either a phenol or a phenolate residue, the interaction is termed a charge-transfer interaction. Such charge-transfer interactions are believed to result in the alteration of the structure of the ligand or the ligand/receptor complex which then activates the receptor. Because such charge-transfer interactions can now be detected by the techniques employed in the present invention, it is now possible to incorporate such interactions into the model created for the naturally occurring biologically active ligand and to create agonists and antagonists to the complementary receptor.

Preferably, when analyzed by nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect (as defined below), the ligand should have a molecular weight of less than about 15,000 daltons, and more preferably, less than about 10,000 daltons, even more preferably, less than about 5,000 daltons and most preferably, less than about 3,000 daltons. However, in the fluorescence analysis of this invention, any molecular weight biologically active ligand can be employed.

"Angiotensin II" -- refers to the biologically active ligand which is an octapeptide represented by the amino acid sequence of

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

wherein each of the above abbreviations are art recognized abbreviations for amino acids.

"Oxytocin" -- refers to the biologically active ligand which is a nonapeptide represented by the amino acid sequence of

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-GlyNH_2

wherein each of the above abbreviations are art recognized abbreviations for amino acids.

"Vasopressin" (arginine vasopressin) -- refers to the biologically active ligand which is a nonapeptide represented by the amino acid sequence of

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-GlyNH_2

wherein each of the above abbreviations are art recognized abbreviations for amino acids.

"Receptor" -- a molecule which binds the ligand.

"Biologically active receptor" -- a molecule, having a specific binding site for its complementary ligand, and can include classical hormone receptors, binding and/or transport proteins, enzymes, antibodies and the like. One embodiment of a biologically active receptor includes membrane bound proteins which control certain cellular processes and which themselves are regulat d by the binding (or lack f binding) f its

complementary naturally occurring biologically active ligand. Because such membrane bound biologically active receptors are bound to membrane, it is believed that the conformation of the biologically active ligand necessary to activate such receptors are lipid induced. See, for instance, Sargent et al., Proc. Natl. Acad. Sci. (USA), 83(16), pp. 5774-5778 (1986) and Surewicz et al., J. Amer. Chem. Soc., 110, pp. 4412-4414 (1988). On the other hand, there are other biologically active receptors which are not membrane bound. In such cases, such receptors may not require a lipid induced conformation of the biologically active ligand and, in fact, may require an aqueous induced conformation of the complementary biologically active ligand in order to activate such receptors.

Examples of biologically active receptors have been well documented in the art. Specific examples include insulin receptor (wherein the complementary ligand is insulin), the V1 receptor (wherein the complementary ligand is vasopressin), the V2 receptor (wherein the complementary ligand is vasopressin), oxytocin-neurophysin (where the complementary receptor is oxytocin), the Angiotensin II receptor (wherein the complementary ligand is Angiotensin II), and the like.

"Agonist" -- A biologically active ligand which binds to its complementary biologically active receptor and activates the latter either to cause a biological response in the receptor or to enhance pre-existing biological activity of the receptor. The agonist can be the naturally occurring biologically active ligand or it can be a synthetic molecule which

can also activate the receptor. For example, it is known in the art that Angiotensin II acts as an agonist for its complementary receptor, the Angiotensin II receptor. Other examples of agonists for the Angiotensin II receptor include [Sar^1]Angiotensin II and the like. Examples of agonists for other receptors include norepinephrine (for its complementary receptor the alpha or beta adrenergic receptors). A common characteristic of all agonists in this invention is that the charge-transfer interaction in the agonist which is necessary to activate the biologically active receptor is not compromised. That is to say that the charge-transfer interaction is operable in the agonist.

"Antagonist" -- A biologically active ligand which binds to its complementary biologically active receptor and either prevents the activation of the latter or deactivates the latter so as to either prevent or diminish the biological activity of the receptor. For example, it is known in the art that the non-peptides 2-n-butyl-1-[4-carboxybenzyl]-4-chloroimidazole-5-acetic acid) and (methyl 2-n-butyl-1-[4-(2-carboxybenz-amido)benzyl]-4-chloroimidazole-5-acetate, sodium salt act as antagonists of the Angiotensin II receptor. See Hypertension, 13, No. 5, May 1989. Other examples of art recognized antagonists to the Angiotensin II receptor include the peptides sarmesin, and the like. Examples of art recognized antagonists to other biologically active receptors include propranolol for the ß-adrenergic receptor, cimetidine for the Histamine-H_2 receptor and the like. A common characteristic of all antagonists in this invention is that the charge-transfer interaction in the antagonist which is nec ssary to activate the

biologically activ receptor is compromis d. That is to say that the charge-transfer interaction in the antagonist is impaired and accordingly, the antagonist cannot activate the complementary receptor. For example, one method of impairing the charge-transfer interaction is to modify the hydroxyl group from the phenol moiety by, for example, methylating, (e.g., forming the $-\phi-O-CH_3$ group). Another method of impairing the charge-transfer interaction is to remove the hydroxyl group from the phenol moiety, e.g., changing phenol to phenyl.

For example and as noted above, Moore et al., Bioscience Reports, 5, pp. 407-416 (1985), proposed the presence of a charge-transfer interaction in Angiotensin II among the C-terminal carboxylate residue, the histidine amino acid and the tyrosine amino acid. In view of this charge-transfer interaction, two classes of antagonists to Angiotensin II are recognized; both of which have an impaired charge-transfer interaction. The first class involves antagonists in which the tyrosine hydroxyl group is modified or deleted and in which the N-terminal amino acid has been modified (e.g., [$Sar^1Tyr(Me)^4$]Angiotensin II, Sarmesin). The other class of antagonists to Angiotensin II involves antagonists in which the C-terminus is modified, with or without concomitant modification of other parts of the molecule (e.g., [Sar^1Ile^8]Angiotensin II, Sarilesin].

Thus, while an antagonist is a biologically active ligand, it is not a biologically active ligand having an active site based on a charg -transfer

interaction because, by definition, this charge-transfer interaction has been impaired.

"Mimetics" -- refers to agonists and antagonists to a biologically active receptor but which have a different structural formula (primary structure) than the naturally occurring biologically active ligand for said receptor. That is to say that mimetics are non-naturally occurring biologically active ligands.

"Tertiary structure of a biologically active ligand" -- refers to the art recognized term which describes the three-dimensional in vivo organization of the individual atoms of such ligands including the charge distribution map so generated. The tertiary structure of a biologically active ligand (often termed its "conformation") reflects non-covalent interactions between/among atoms as well as covalent bonding between atoms. Non-covalent interactions include both electrostatic and non-electrostatic interactions such as ionic bonds, hydrogen bonding, Van der Waal forces, etc. Because the extent and nature of such non-covalent interactions are dependent on the polarity of the solvent in which they are measured, the tertiary structure (conformation) of such ligands will change when taken from its in vivo micro-environment and placed into an environment of different polarity.

"Fluorescence compatible environment" -- is an environment where long lifetime fluorescence (LLF - defined hereinbelow) can be detected. In this regard, it is noted that certain solvents such as dimethylsulfoxide (DMSO) and water do not permit detection of LLF, presumably because of such factors as

solvent induced fluorescenc qu nching, solvent interference with intramolecular hydrogen bond formation. On the other hand, the use of aqueous solutions of micelles and lipid bilayers as well as the use of solvents having a dielectric constant of about 40 or less allows for detection of LLF. Preferably, solvents having a dielectric constant of less than 40 are employed as the fluorescence compatible environment. Even more preferably, the dielectric constant fluorescence compatible environments is from about 2 to about 40. Suitable solvents having a dielectric constant of about 40 or less include, for instance, propylene glycol, isopropanol, trifluoroethanol and the like. Lastly, the solvent so selected should itself not possess fluorescence in the region where the LLF is being detected.

"Receptor-simulating environment" -- refers to an environment created to simulate the polarity of the in vivo micro-environment in the immediate vicinity of a biologically active receptor. As noted above, if a biologically active ligand is placed into an environment of different polarity from its in vivo micro-environment, its tertiary structure will change but not its structural formula, i.e., the covalent bonds will not change. The addition of a biologically active ligand into a receptor-simulating environment allows the ligand to substantially conform to the tertiary structure it would possess if placed in the micro-environment of its complementary biologically active receptor. For example and as noted above, for membrane bound biologically active receptors, it is believed that the conformation of a biologically active ligand responsible for activating the recept r is lipid

induced. Accordingly, for such receptors, the receptor-simulating environment will be less polar than aqueous environments and solvents having a dielectric constant of about 50 or less have been found to provide a receptor-simulating environment for such membrane bound receptors. Suitable solvents having a dielectric constant of about 50 or less include dimethylsulfoxide (DMSO), trifluoroethanol, isopropanol, propylene glycol, and the like. For non-membrane bound receptors, a solvent having a dielectric constant of about that of water or less will provide a receptor-simulating environment.

"Three-dimensional spatial model of a biologically active ligand" -- refers to the tertiary structure of such a biologically active ligand created from the analytical techniques herein described. The creation of such three-dimensional spatial models is sometimes referred to herein as "modelling".

Because the NMR techniques which are employed to create the three-dimensional spatial model employ a receptor simulating environment, the model created will substantially conform to the biologically active ligand's tertiary structure. However, because the polarity of the solvent simulating enviroment will not be exactly the same as the in vivo micro-environment, the three-dimensional model will possess minor variations from the tertiary structure. Provided that a receptor simulating environment is employed, the resulting variations will be minor in nature and the three-dimensional spatial model will provide meaningful information concerning th in vivo t rtiary structur of the bi logically active ligand.

"NMR spectroscopy using the Nuclear Overhauser effect" -- refers to the nuclear magnetic resonance methodology which permits insights into the three-dimensional spatial organization of the ligand's atoms. Suitable NMR methodologies include proton [^{1}H] NMR, ^{19}F NMR, ^{13}C NMR, and the like. Preferably, proton NMR is employed.

The first step of this methodology employs Correlated Spectroscopy ("COSY") which is a two-dimensional NMR spectrum yielding information on through-bond coupling patterns within a molecule. COSY methodology permits the assignment of individual proton resonances within the spectrum to particular protons in the ligand. This information is then used to identify NOE correlations. Such COSY methodology is well known in the art and is described by Cheatham, Journal of Chemical Education, 66, pp. 111-117 (1989). In some cases, COSY methodology can be supplemented by ROESY and 1-D NOE methodologies in the assignment of individual proton resonances within the spectrum to particular protons in the ligand.

Once the two-dimensional assignments have been made via the COSY methodology, the next step is to conduct nuclear magnetic resonance (NMR) employing the nuclear Overhauser effect methodology [such as one-dimensional NOE enhancement, two-dimensional NOESY and two-dimensional ROESY (rotating frame nuclear Overhauser effect spectroscopy)] on the ligand. NMR employing the nuclear Overhauser effect methodology is used to describe a change in intensity of one NMR line when another line is irradiated at the frequency of the latter line. The chang in intensity is due to

"through space" energy transfer from one atomic nucleus to another. Thus, the nuclear Overhauser effect provides information of nearest neighbor atomic nuclei to the line that is saturated. Accordingly, the accumulation of a sufficient number of the nuclear Overhauser effects among neighboring atoms can be used to determine the spatial characteristics for the entire molecule.

The nuclear Overhauser effect is a well known and art recognized NMR effect and is described by Cheatham, Journal of Chemical Education, 66, pp. 111-117 (1989). This reference describes the use of one-dimensional NOE enhancement as well as the use of NOE in 2-dimensional NMR (NOESY) as a tool to create three-dimensional models. The ROESY method is also well known and art recognized and is described by Bax and Davis, J. of Magn. Reson., 63, pp. 207-213 (1985). The use of ROESY is particularly suitable for intermediate size molecules such as peptide hormones.

"Fluorescence analysis" -- refers to the identification of a charge-transfer system in a biologically active ligand by using a fluorescence instrument capable of measuring fluorescence decay at the level of a nanosecond, or shorter, time intervals. Such equipment is known in the art and is commercially available, for example, from Photochemical Research Associates under the tradename System 3000. Fluorescence decay due to an excited-state phenol (or phenolate) species involved in the charge-transfer interaction is determined in a fluorescence compatible environm nt after excitation with light of a suitable wav length. For exampl , if tyrosine is involv d in

the charge-transf r interaction s as to result in a tyrosinate species, fluorescence decay due to excited-state tyrosinate emitting at and around 350 nm is determined after excitation with light of a suitable wavelength, e.g., 275 nm. Other excited state species (e.g., ligands with phenol containing groups other than tyrosine) involved in the charge-transfer interaction can also be determined by measuring their fluorescence decay at a suitable wavelength after excitation at an appropriate wavelength. The appropriate wavelengths of absorption and emission can be readily determined by the skilled artisan for any given phenol containing ligand.

The experimentally obtained fluorescence decay, which is described as a sum of exponentials, is deconvoluted, and the lifetime of the longest component due to the phenolate species of interest is determined. Methods for summing the exponentials to obtain the fluorescence decay, deconvolution of the fluorscence decay and determining the lifetime of the longest component due to the phenolate species are known in the art and exemplified in the examples set forth hereinbelow.

Long lifetime fluorescence ("LLF") -- is the half-life of the longest living fluorescent component emitting at or around the species' fluorescent maximum and is employed to determine the existence of a stable charge-transfer interaction occurring in the ligand. In particular, in tyrosinate excited-state fluorescence analysis in propylene glycol, LLFs greater than about 11 nanoseconds and preferably greater than about 12 nanoseconds are diagn stic that th tyrosinate moiety

or modified tyrosinate moiety is participating in a stable charge-transfer interaction. Such diagnosis is made on the basis that LLF's greater than about 11 nanoseconds for tyrosine or modified tyrosine containing ligands in propylene glycol correlate to the presence of at least some (i.e., ≥1% relative to Angiotensin II) agonist activity for said ligands. On the other hand, a LLF of 11 nanoseconds or less in propylene glycol is indicative that the tyrosinate species or modified tyrosinate species responsible for the LLF is not sufficiently stable and does not activate the receptor. Again, such diagnosis is made on the basis that LLFs of about 11 nanoseconds or less for tyrosine or modified tyrosine containing ligands correlate to inactive or antagonist activity for said ligands (agonist activity of less than 1% relative to Angiotensin II). Similar correlations to determine whether a species different from tyrosine in a ligand is participating in the charge-transfer interaction can be made based on the LLFs of this species or modified species in a variety of ligands correlated to whether the particular ligand is an agonist, is an antagonist or is inactive.

Without being limited to any theory, it is believed that the charge-transfer interaction imparts a level of stability to the excited state of the species (e.g., tyrosine), which permits a longer LLF for the species. Accordingly, longer LLFs correlate to the presence of a charge-transfer interaction which in turn correlate to agonist activity.

Having d fined the terms used herein, the inv ntion will now be described in detail.

As noted abov , the first step in the preparation of a three-dimensional spatial model of a biologically active ligand having one or more charge-transfer interactions is a fluorescence analysis of the biologically active ligand. That is to say that the ligand is analyzed using fluorescence techniques in order to determine the existance of a charge-transfer interaction. In the following description of this fluorescence technique, Angiotensin II will be employed as a representative ligand. However, it is understood that other biologically active ligands can be analyzed in the same manner as Angiotensin II by using the methods hereinbelow described for Angiotensin II.

Nanosecond time-resolved fluorescence decays of Angiotensin II and analogs thereof were measured by taking advantage of the characteristic fluorescent properties of the excited-state tyrosinate species (other phenolate species would also exhibit similar characteristic properties for their excited-state). In this regard, in order for fluorescence emission from tyrosinate (and other phenolate species) to occur, there must be proton transfer to/from the phenolic hydroxyl group from/to an appropriate acceptor group. Based on the pKa's of tyrosine in the ground state (10.4) and in the excited state (less than or equal to about 5.4), protolysis in the excited-state is more efficient.

In particular, nanosecond time-resolved fluorescence decays of Angiotensin II and analogs thereof were measured from the emission at 350 nm due to its excited-state. Long lifetime fluorescence (LLF) was determined for each of th se analogs in several

solvents of different polarity using N-acetyl-tyrosine-amide as the reference standard. The results of this analysis demonstrate that solvents such as water and DMSO do not allow detection of long lifetime fluorescence in these analogs; presumably because of factors such as solvent induced fluorescence quenching, solvent interference with intramolecular hydrogen bond formation, etc. On the other hand, use of a fluorescence compatible environment such as aqueous lipid bilayer solutions, micelles in an aqueous environments, and solvents having a dielectric constant of about 40 or less permit the detection of long lifetime fluorescence.

Without being limited to any theory, it is believed that this detection of the long lifetime fluorescence in a fluroescence compatible environment is due to the fact that such environments either do not quench the fluorescence generated by the tyrosinate excited-state and/or do not interfere with intramolecular hydrogen bonding in Angiotensin II. Additionally, as noted above, that Angiotensin II conformation (tertiary structure) which permits the formation of a charge-transfer interaction will stabilize the tyrosinate excited-state which in turn results in very long lifetime fluorescence. Insofar as the conformation of Angiotensin II is not stagnant but in fact is dynamic (i.e., in a given environment at a given temperature, Angiotensin II is constantly changing conformation both <u>in vitro</u> and <u>in vivo</u>), only that conformation which permits formation of the charge-transfer interaction responsible for receptor activation will result in the formation of a very long lifetime fluorescenc . Accordingly, the environm nt

used for determining the presence of a charge-transfer interaction via such fluorescence analysis should be selected to be compatible with the fluorescence analysis and to allow for the presence of that conformation which permits this interaction. Such results are achieved with the fluorescence compatible environment employed in this invention. Preferably, the fluorescence compatible environment will maximize the presence of that conformation of such a biologically active ligand which actives the receptor; but such is not necessary provided that the fluorescence compatible environment permits the presence of a sufficient amount of the conformation of the biologically active ligand which activates the receptor so that its LLF can be detected.

In membrane bound receptors, recent evidence from site-specific receptor mutation studies suggests that small ligands, i.e., ligands having a molecular weight of less than about 3,000 daltons, bind to a site in one of the transmembrane domains of the receptor protein and therefore may have a biologically active conformation which is lipid-induced. In such cases, it is believed (again without being limited to such a theory) that use of solvents of intermediate polarity or less (i.e., having a dielectric constant of about 50 or less), lipid bilayers and micelles provides a receptor environment which simulates the micro-environment which the ligand encounters in the vicinity of such membrane bound receptors. Thus use of a fluorescence compatible environment for biologically active ligands complementary to such receptors provides the additional advantage that such environments should

facilitate the maximization of the ligand's conformer responsible for activating the receptor.

Table I below shows the average long lifetime fluorescence values obtained from Angiotensin II and related analogs in isopropanol as well as propane-1,2-diol (propylene glycol). Table I also shows the agonist activity of Angiotensin II as well as for the listed analogs. [The data set forth in Table I below was obtained in a manner similar to that set forth in Examples 1 and 3 set forth hereinbelow].

TABLE I

LIGAND	SOLVENT: PROPANE-1,2-DIOL LLF[a]	% LLF	SOLVENT: ISOPROPANOL LLF[a]	% LLF	AGONIST ACTIVITY[b]
A	20.8	19	15.5	79	100
B	13.1	46	13.1	10	27
C	18.8	11	9.3	11	7
D	14.9	13	0	--	4[c]
E	16.2	10	0	--	5[c]
F	9.2	6	11.6	3	0.2
G	6.6	35	0	--	less than 0.1
H	10.6	17	9.4	16	less than 0.1
I	10.2	8	8.5	12	less than 0.1
J	0	--	6.5	20	less than 0.1[d]
K	7.4	10	10.2	14	10

a = in nanoseconds
b = Agonist Activity was measured via a rat isolated uterus bioassay as described by Matsoukas et al., J. Med. Chem., 31, pp. 1418-1421 (1988). Results are reported relative to Angiotensin II wherein Angiotensin II = 100.
c = Potent receptor antagonist with residual agonist activity.
d = Potent receptor antagonist.

Ligand A = Angiotensin II
Ligand B = [$Sar^1His(3\text{-}Me)^6$]Angiotensin II
Ligand C = [Sar^1Phe^6]Angiotensin II
Ligand D = [Sar^1Cha^8]Angiotensin II
Ligand E = [Des^1Cha^8]Angiotensin II
Ligand F = [$Sar^1Phe\text{-}NH_2{}^8$]Angiotensin II
Ligand G = [Sar^1Ala^6]Angiotensin II
Ligand H = [$Sar^1His(1\text{-}Me)^6$]Angiotensin II
Ligand I = [$Sar^1D\text{-}Pro^7$]Angiotensin II
Ligand J = [Sar^1Ile^8]Angiotensin II (Sarilesin)
Ligand K = Angiotensin III

The preparation of Ligands B-K is well known in the art. See, for instance, Matsoukas et al., Journal of Med. Chem., 31, pp. 1418-1421 (1988).

Sar = sarcosine
Cha = cyclohexylalanine
Des = amino acid residue omitted

In Table I above, % LLF measures the percent of conformer(s) present which give rise to LLF.

The above data demonstrate that strong agonists, Ligands A and B, possess a long lifetime fluorescence in isopropanol of greater than 13 nanoseconds as compared to Ligands possessing either low agonist activity, antagonist activity or inactivity, Ligands C-K. Likewise, in propylene gylcol, Ligands (except Angiotensin III) possessing any agonist activity, Ligands A-E, possess a long lifetime fluorescence of greater than 11 nanoseconds, whereas Ligands either possessing no activity or antagonist activity without any residual agonist activity, Ligands F-J, possess a long lifetime fluorescence of 11 nanoseconds or less. Accordingly, prolonged duration of the long lifetime fluorescence correlates to agonist activity which in turn indicates that tyrosine's phenol residue is involved in the charge-transfer interaction responsible for receptor activation.

Contrasted with the readily conducted method of this invention which establishes that tyrosine is involved in the active state of Angiotensin II via fluorescence analysis, the prior art had previously determined that the tyrosine hydroxyl group of Angiotensin II played an important role in receptor activation either by preparing Angiotensin II analogs without tyrosine or by methylating the hydroxy group of tyrosine. It is clear that the process of the present invention is more facile and does not require the synthesis of numerous analogs of Angiotensin II. M reover, modification of Angiotensin II by removal of amino acids etc., can in fact change th tertiary

structure of the analog r lative to Angiotensin II such that meaningful conclusions may be difficult to reach.

Once a charge-transfer interaction has been identified in the ligand via the fluorescence analysis of this invention, the next step in the process of preparing a three-dimensional spatial model of a biologically active ligand having one or more charge-transfer interactions is a determination of the chemical groups involved in the charge-transfer interaction. Such a determination can be conducted by using art recognized structure activity relationships. In this regard, these determinations are greatly facilitated by the knowledge that a phenol/phenolate species is involved in the charge-transfer interaction. Accordingly, in those ligands having only one such species (e.g., tyrosine) it is readily apparent that such a species is involved in the charge-transfer interaction.

In general, structure activity relationships are conducted by creating analogs of the ligand of interest by selectively replacing or modifying one of the components of the ligand (e.g., in the case of a peptide, an amino acid), and then determining the LLFs of the analogs. Reduction in the LLF of an analog as compared to the ligand is significant evidence that the component originally found in the ligand and subsequently replaced or modified in the analog plays a role in the charge-transfer interaction. See Ligands F and G in Table I which identify the histidine and C-terminal carboxylate in the charge-transfer interaction in Angiotensin II. Additionally, loss of agonist activity in the analog provides corroborating evidence

that the component plays a role in the charge-transfer interaction. In this regard, if the ligand contains two or more phenol/phenolate species, determination which of such species are involved in the charge-transfer interaction can be made by creating analogs in which one of the two or more phenolic groups has been compromised, by for example, methylating the hydroxy group. Analysis of the LLFs and biological activities of such analogs will provide the required information to determine which of the two or more phenolic groups is involved in the charge-transfer interaction.

Once the groups involved in the charge-transfer interaction have been identified, the next step in the process of preparing a three-dimensional spatial model of a biologically active ligand having one or more charge-transfer interactions is to resolve remaining aspects of the ligand's three-dimensional spatial conformation by obtaining conformational information relative to the active site from nuclear magnetic resonance spectrscopy employing the nuclear Overhauser effect.

As noted above, this step first involves the use of COSY methodology which provides information on through-bond coupling patterns within a molecule and allows for the two-dimensional assignment of individual protons in the ligand. The COSY methodology is established in the art. After the two-dimensional assignment of the individual protons via COSY methodology, the ligand is then examined by conducting nuclear magnetic resonance employing the nuclear Overhauser effect m thodology. Suitable nuclear Overhaus r effect methodologies include one-dimensional

NOE enhancement, two-dimensional NOESY and two-dimensional ROESY. All of these nuclear Overhauser effect methodologies are established in the art.

However, with regard to ligands having a molecular weight of between about 500 to 2000 daltons, the use of NOESY methodology often fails for such ligands, irrespective of the internuclear distances involved, because the tumbling rate for these solutes is close to that at which the maximum possible NOE passes through zero. See Bax and Davis, J. Magn. Reson., 63, pp. 207-213 (1985). Consequently, sequential assignments and the observation of interproton distances revealing structures are impossible using NOESY for such ligands. However, such ligands can be structurally analyzed using either one-dimensional NOE enhancement or ROESY methodologies.

In further regard to nuclear magnetic resonance spectroscopy using the nuclear Overhauser effect, there is a practical limit on the molecular weight of the ligand being analyzed. In particular, ligands having a molecular weight of about 15,000 daltons or greater impose to much complexity on current NOESY/ROESY methodologies to permit their use. However, in certain circumstances, one-dimensional NOE methodologies could be used. Accordingly, in this invention, ligands being investigated by nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect preferably have a molecular weight of less than about 15,000 daltons and preferably have a molecular weight of less than about 10,000 daltons.

The solvents used when conducting proton nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect are selected so as to provide a receptor simulating environment. Thus, if the biologically active receptor is a membrane bound receptor, current hypotheses suggest the role of lipid-induced peptide folding in peptide hormone-receptor interactions. See Sargent et al., Proc. Natl. Acad. Sci. (USA), 83(16), pp. 5774-5778 (1986); and Surewicz et al., J. Amer. Chem. Soc., 110, 4412-4414 (1988). Such lipid-induced peptides are generally believed to have a molecular weight of less than about 3,000 daltons. Thus, in these circumstances, the use of solvents having a dielectric constant of about 50 or less is justified. Furthermore, such dielectic constants allow for a more ordered peptide structure.

A particularly preferred solvent for use in nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect for ligands whose complementary receptor is a membrane bound receptor is dimethylsulfoxide (DMSO). In particular, DMSO is preferred because it offers several advantages over other possible solvents having a dielectric constant of about 50 or less for the following reasons: 1) the solvent allows for the buildup of NOEs to a level of detectability which is not possible in solvents such as deuterated water; 2) for the reasons noted above, the bulk dielectric environment provided by DMSO is such that it represents an environment not unlike that encountered by such peptides at their receptors, and which gives useful and practical information; 3) the spectra are characterized by sharp and well resolved proton signals which can be individually assigned using

COSY methodology and are often superior to spectra obtained in solvents such as trifluoroethanol, propylene glycol and isopropanol which give broader and often overlapping signals; 4) DMSO is superior to aqueous environments for charged molecules because fewer conformations are usually sampled and conformational averaging is altered in DMSO as compared to water; and 5) the dielectric constant of DMSO (-45) is sufficiently close to the maximum dielectric constant employed in the fluorescence analysis so that minimal conformational changes are expected in the two enviroments.

When the receptor simulating environment is aqueous in nature, the use of water or a solvent mixture containing water is justified.

In any event, when the NMR methodologies described hereinabove are proton [^{1}H] NMR methodologies, deuterated solvents will be required, i.e., d_6-DMSO, D_2O and the like.

Examples 4-6 hereinbelow set forth biologically active ligands which have been analyzed by nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect. In this regard, Examples 1-3 had already established that the tertiary structures for naturally occurring biologically active ligands (i.e., [Sar^1]Angiotensin II and oxytocin) employ a charge-transfer interaction to activate the biologically active receptor and which groups were involved in the charge-transfer interaction.

Further in this regard and by using the methods of the present invention, molecular models of biologically active ligands have been developed. In particular, FIGURE 1 illustrates a molecular model of [Sar^1]Angiotensin II. In FIGURE 1, the backbone of [Sar^1]Angiotensin II is maintained by two gamma turns maintained in part by hydrogen bonds between the Arg CO and Tyr NH and between His CO and Phe NH (not shown). [All of the molecular models depicted herein were developed using Minit Molecular Models, Cochranes, Oxford, U.K. A person skilled in the art can readily reproduce such models.]

For comparison purposes, FIGURES 2A, 2B and 2C illustrate simplified two-dimensional structures showing some conformational aspects of Sarmesin, Angiotensin II and Sarilesin, respectively. In reality, the aromatic rings lie above the peptide backbone. See FIGURES 1 and 4A.

FIGURE 4A illustrates a molecular model of Angiotensin II determined in DMSO/D_2O by 2D-ROESY proton NMR in a manner similar to that of Example 4. The backbone of Angiotensin II is characterized by two gamma turns maintained in part by hydrogen bonds between the Arg CO and Tyr NH and between His CO and Phe NH (not shown). FIGURE 6 illustrates three-dimensional stereo photographs of the model of Angiotensin II. In regard to the figures containing stereo photographs, it is noted that such photographs should be viewed by stereo glasses/viewer in order to obtain th three-dimensional effect. Such st reo glasses/view rs ar commercially availabl ;

one source being Marivac, 1872 Garden St. Halifax, Nova Scotia, B3M 3RL, Canada.

Once a three-dimensional spatial model for a biologically active ligand has been developed using the techniques of this invention, further refinement of this model or development of even new models can be accomplished using theoretical considerations. For example, with knowledge of the three-dimensional model for Angiotensin II depicted in FIGURE 4A and illustrated in the stereo photographs of FIGURE 6, it is possible by employing theoretical considerations to create a three-dimensional model for Angiotensin II bound to its receptor, the Angiotensin II receptor. In particular, such theoretical considerations generally relate to readily available chemical pathways. For instance, because of the charge-transfer interaction, the tyrosine hydroxyl group in Angiotensin II has been converted to its tyrosinate species. The tyrosinate species, which is a strong nucleophile, can then be derivatized by the receptor resulting in transient bonding between the ligand and the receptor. Upon such bonding, tyrosine moves away from the histidine side chain because the histidine is no longer able to form a hydrogen bond with the tyrosine hydroxyl group. Moreover, slight repositioning of the histidine is also expected. Such theoretical considerations have already been forwarded. See, for instance, Moore et al., Int. J. Pept. Prot. Res., 26, pp. 469-481, (1985). In view of the above, a receptor bound three-dimensional spatial model of Angiotensin II was developed which accounts for such conformational changes which would occur if Angiotensin

II behaves in the suggested manner. A stereo photograph of this model is depicted in FIGURE 7A.

The validity of the resulting model can be readily verified by overlaying known antagonists onto the receptor bound model and ascertaining whether the antagonists can conform to the model. That is to say that if the model is correct, then the antagonists should be able to adapt a conformation similar to the model of the ligand so as to bind to the receptor and thereby account for their antagonist behavior. In this regard, FIGURES 3A and 3B illustrate examples of compounds from a class (i.e., structurally related compounds) of antagonists of Angiotensin II. This class is generically known as either N-benzyl-imidazole compounds ("BI") or N-benzamidobenzyl-imidazole compounds ("BABI"). It is noted that in FIGURE 3B, the acidic proton in BABI is present in a hydrogen bonded form (depicted by the box around this proton together with the dots to the amido carbonyl group) somewhat analogous to the hydrogen bonded form of the tyrosinate species of Angiotensin II. Many acidic groups can exist in similar hydrogen-bonded stabilized forms in BABI compounds, e.g., carboxylate (shown), sulfate, trifluoro-methylsulfonamido, and the like. It is also noted that for the BI class of compounds, the acidic proton can occupy a similar position in space to the acidic proton shown in BABI, but that the former is not stabilized by hydrogen bonding.

In FIGURE 5B, the common portion of these antagonists have been overlayed onto the imidazol portion of Angiotensin II depicted in FIGURE 5A which additionally shows the relative position of th

compon nts responsible for the charge-transfer interaction in Angiotensin II (note--the imidazole double bonds have been removed from FIGURE 5B for the sake of clarity). In FIGURE 5B, the fact that the hydroxyl group of the hydroxymethyl in both antagonists is similarly located to the tyrosine hydroxy group in Angiotensin II; the fact that the n-butyl side chain of both antagonists mimics precisely the His C_β-His C_α-His CO-Pro N chain of Angiotensin II; and the fact that the chlorine atom in these antagonists can serve to decrease the basicity of the imidazole nucleus of these compounds, indicates that the antagonist can form a conformation with similar electronic and three-dimensional characteristics as the conformation of Angiotensin II required for generation of the charge-transfer interaction responsible for activating the receptor. However, because these antagonists lack the necessary functionality to generate a charge-transfer interaction, they can not activate the receptor which accordingly explains their antagonist properties.

Having generated a model for the tertiary structure of a biologically active ligand, it is now possible to design and synthesize mimetics to this ligand. For example, it is now possible to design and synthesize compounds which are sufficiently similar to the model generated for the tertiary structure of the biologically active ligand so as to be complementary to the ligand's receptor. In this regard, antagonists are created when the compound so designed and synthesized has a compromised charge-transfer interaction whereas agonists are created when the compound so designed and synthesized has an operable charge-transfer interaction, i.e., the charge-transf r interacti n is

not impaired. With knowledge of the model generated for the tertiary structure of a biologically active ligand, the design and synthesis of agonists and/or antagonists to the ligand's complementary receptor is well within the ability of the skilled artisan.

The present invention also offers a particular advantage in the design and synthesis of new mimetics optionally based on the structure of known mimetics coupled with knowledge of the model generated for the tertiary structure of a biologically active ligand. This particular advantage is especially applicable to designing new mimetics of Angiotensin II, which may or may not be based on the structure of known mimetics.

In particular, structure-activity relationships show that the binding affinity between Angiotensin II and its complementary receptor derives largely from Coulombic [ionic] forces originating from complementary charges between Angiotensin II and its receptor. The ionic charges on Angiotensin II are illustrated in FIGURE 4B which is based on the model for Angiotensin II depicted in FIGURE 4A. In FIGURE 4B, N˙ denotes tyrosinate which relocates upon interaction with the receptor (this is shown in FIGURE 7A which is a stereo photograph of receptor bound Angiotensin II). On the other hand, the N-benzyl-imidazole (BI) and N-benzamidobenzyl-imidazole (BABI) class of known antagonists to Angiotensin II [Wong et al., Hypertension, 13, pp. 489 et seq., (1989)] are devoid f many of the charges which cause Angiotensin II to bind tightly to its rec ptor. Ov rlay of th imidaz le group f the BI and BABI compounds d pict d

in FIGURES 3A and 3B onto the imidazole group of the model for Angiotensin II depicted in FIGURE 5A is illustrated in FIGURE 5B. The three-dimensional organization of the chemical groups of BI and BABI compounds is such that these compounds can mimic 1) Angiotensin II, 2) Sarmesin, or 3) Sarilesin. (For example, when the imidazole-based hydroxyl group of BABI compounds is methylated, the resulting oxymethyl group occupies a similar position in space to the oxymethyl group of Sarmesin.) Overlay of a specific BABI compound (depicted in FIGURE 7C) onto the receptor bound model of Angiotensin II (depicted in FIGURE 7A) is illustrated in the stereo photograph of FIGURE 7B. As can be seen from FIGURE 7B, because the BABI com-pound has a similar spatial arrangement to the Tyr-Val-His sequence of Angiotensin II (as well as to Sarilesin), the BABI compounds can mimic this portion of the model of Angiotensin II (and Sarilesin) so as to be complementary to the Angiotensin II receptor. Further in this regard and without being limited to any theory, it is believed that the receptor may transiently acylate, or the like, the tyrosine hydroxyl group of receptor bound Angiotensin II and alter the location of the tyrosine side-chain relative to its position in the "charge-transfer interaction" form of Angiotensin II. Moreover, it is further believed that the acidic portion of BABI compounds which is stabilized in a predisposed or "preactivated" form by a hydrogen bonding interaction with the carbonyl oxygen of the amido group (See FIGURE 3B), occupies a position in space which is similar to that of the tyrosine hydroxyl group in the receptor bound model for Angiotensin II depicted in FIGURE 7A.
It is still further b lieved

that a bond, similar to that formed between the "preactivated" tyrosinate group of Angiotensin II and a receptor-based acceptor group, will also be formed between the "preactivated" acid group of BABI compounds and the receptor. In contrast to Angiotensin II, it is also believed that for the case of BABI compounds, this will not result in receptor activation because of the different conformational constraints and the nature of the ligand-receptor bond. Thus, for example, if the receptor acylates the tyrosine OH group of Angiotensin II, the adduct formed between Angiotensin II and its receptor will involve an ester bond, whereas that for the BABI compound shown in FIGURE 3B will involve an anhydride linkage or for the BABI compound shown in FIGURE 7C will involve an amide linkage. This discloses the fact that BABI compounds are Angiotensin II receptor antagonists because they can act as transition state inhibitors or suicide substrates for the Angiotensin II receptor. In contrast to BABI compounds, BI compounds are not likely to act by this mechanism because the acidic proton is not stabilized by hydrogen bonding, and is therefore not preactivated.

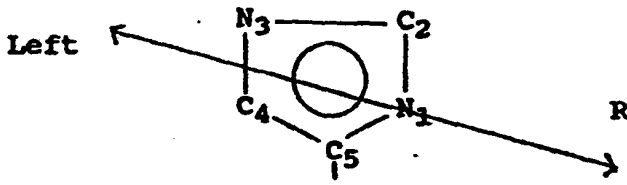
In any event, it is seen that the BI/BABI class of compounds commonly possess an imidazole ring which can be modified to enhance the potency of these compounds.

This information, in conjunction with the charge distribution map depicted in FIGURE 4B, allows for the design and synthesis of new antagonists to the Angiotensin II receptor based on incorporating additional charges at th appropriat location into BI and BABI compounds so as to increase the binding

affinity of these antagonists to the Angiotensin II receptor and accordingly increase their potency. In view of the above, derivatives of the BI and BABI compounds having one or more such charges can be prepared as follows [from FIGURE 4B, it can be seen that all charges (except the tyrosinate charge) including the imidazole ring lie in the same approximate plane:

1. All distances are given relative to the center of planar imidazole ring (either of the His amino acid in Angiotensin II or of the imidazole ring of the BI/BABI compounds).

2. The placement of the charges is defined by a line drawn through the center point of the imidazole ring which bisects the N_3-C_4 bond of imidazole ring of histidine as shown in Formula I as follows:



I

wherein the subscripts 1-5 correspond to accepted numbering of a histidine imidazole ring. [For analogs in which the His ring is rotated through 180°, e.g., His(3-methyl) analogs, the bisected bond becomes the N_1-C_2 bond.]

3. One or more of the following charges can be placed onto the imidazole ring:

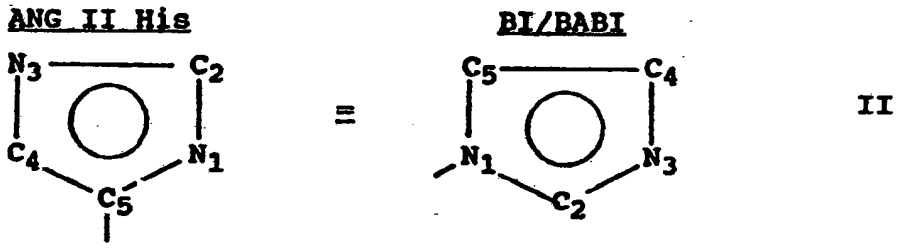
i) Direction: Left
Charge: Cationic
Distance from center of imidazole ring: 7 ± 1.5 Angstroms
(Corresponds to N-terminus cationic charge);

ii) Direction: Right
Charge: Anionic
Distance from center of imidazole ring: 2.5 ± 0.5 Angstroms
(Corresponds to C-terminus anionic carboxylate charge);

iii) Direction: Left
Charge: Anionic
Distance from center of imidazole ring: 10 ± 2 Angstroms
(Corresponds to aspartic acid anionic charge); and

iv) Direction: Left
Charge: Cationic
Distance from center of imidazole ring: 12 ± 2.5 Angstroms
(Corresponds to arginine cation)

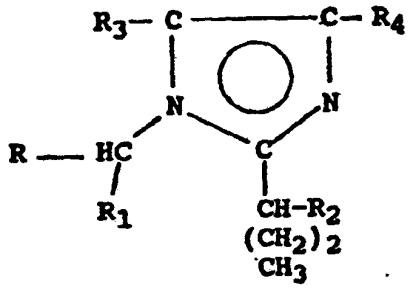
4. In the above, the orientation of the imidazole rings of Angiotensin II and the BI/BABI compounds is as shown in Formula II as follows:



II

Thus C_4 in Angiotensin II is equivalent to N_1 in the BI/BABI class of compounds.

Examples of side chains which can be added to BI/BABI compounds include for instance compounds of the following Formula III:



III

wherein R is selected from the group consisting of a) phenyl para substituted with a substituent selected from the group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido, and b) $-NHC(O)R_5$ wherein R_5 is phenyl ortho substituted with a substituent selected from the group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido, R_1 is either hydrogen or hereinafter defined, R_2 is either hydrogen or as hereinafter defined, R_3 is either hydroxymethyl, $-CH_2OCH_3$, $-CH_2C(O)OCH_3$, $-C(O)OCH_3$, or as hereinafter defined, and R_4 is either fluorine, chlorine or as hereinafter defined.

In view of the above, a compound mimicking the N-terminal cationic charge can be prepared by attaching a suitable amino group at the appropriate location on Formula III. Such a group could be placed at the appropriate distance from the center of the imidazole nucleus by employing an alkyl amino substituent (or another suitable cationic group such as a guanidino group, and the like) wherein the number of

methylene groups employed in the chain linking the amino group to the BI/BABI compound is selected so as to provide a positive charge at 7 ± 1.5 Angstroms left from the center of the imidazole ring. For example, placement at RR_3 of a $-(CH_2)_4-NH_2$ group will provide such a charge (the amino group will protonate in the in vivo environment to form a $-NH_3^+$ group). Likewise, if hydroxyl functionality is to be maintained at R_3, then R_3 will be the group $-CHOH-(CH_2)_3-NH_2$. Alternatively, the positive charge at 7 ± 1.5 Angstroms can be obtained by placement at R_1 of a $-(CH_2)_3NH_2$ group. In still another alternative, R_1 or R_3 can be $-(CH_2)_n$-Asp-Arg-NH_2 wherein n is 3 for R_1 and 4 for R_3 which provides for the 3 charged groups found in the N-terminal dipeptide of Angiotensin II. In regard to the above, only one of R_1 and R_3 should be substituted at any one time with a cationic group.

A C-terminal anionic mimetic can also be prepared by placing a negative charge at right 2.5 ± 0.5 Angstroms to the center of the imidazole ring. For example, placement at R_4 of a $-(CH_2)_3C(O)OH$ group will provide the necessary negative charge at right 2.5 ± 0.5 Angstroms (the carboxyl group will deprotonate in vivo to provide a carboxylate group, i.e., $-C(O)O^-$. Alternatively, placement at R_2 of a $-(CH_2)_3C(O)OH$ will provide the necessary negative charge at right 2.5 ± 0.5 Angstroms. In regard to the above, only one of R_2 and R_4 should be substituted at any one time with an anionic group.

Similar considerations regarding the attachment of charges can be applied to the imidazol group of the His amino acid so as to arrive at mimetics

to the Angiotensin II receptor. This is particularly true because as indicated above, all of the ionic charges in receptor bound Angiotensin II (except the tyrosinate anion) are in approximately the same plane and moreover, in approximately a straight line. Moreover, the imidazole ring is planar and lies in the same approximate plane as the ionic charges. Accordingly, the substituents set forth above for the imidazole group of BI/BABI compounds could be placed at their equivalent points on the imidazole of the His amino acid.

Similar groups can be designed for the aspartic acid anionic charge and for the arginine cationic charge.

The compounds depicted above can be readily prepared by the skilled artisan using art recognized techniques. Such compounds and their pharmaceutically acceptable salts are useful as Angiotensin II antagonists. Accordingly, such compounds can be used to control hypertension and/or congestive heart failure in a mammal in need of such treatment. Additionally, the compounds of this invention are contemplated as being useful in other cardiovascular and related diseases such as stroke, myocardial infarction and the like. When used to control hypertension and/or congestive heart failure, the compound is normally administered to such a mammal either orally or parenterally. When so administered, the compound is generally formulated in a pharmaceutically acceptable diluent and at a dosage sufficient to control hypertension and/or congestive heart failure in the mammal so treated. The specific dos levels for such

uses can be readily determined by the skilled artisan. Accordingly, the present invention contemplates a method for controlling hypertension in a mammal in need of such treatment which comprises either administering orally or parenterally a pharmaceutical composition of a compound depicted above in an amount sufficient to control hypertension. Additionally, the present invention also contemplates a method for treating congestive heart failure in a mammal in need of such treatment which comprises either administering orally or parenterally a pharmaceutical composition of a compound depicted above in an amount sufficient to control said heart failure. The methods of controlling hypertension are implemented using pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an amount of a compound depicted above effective to control hypertension in a mammal in need of such treatment. The methods of controlling congestive heart are implemented using pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an amount of a compound depicted above effective to control said heart failure.

The present invention will be described in further detail with reference to the following examples. However, it should be understood that the present invention is by no means restricted by these specific examples.

EXAMPLES

A. Examples 1 and 3 below are directed to the identification by fluorescence analysis of the presenc r absenc of a tyrosinat charge-transfer interaction in biologically active ligands. The

fluorescence analysis was measured on a nanosecond, or shorter, time intervals. Fluorescence decay due to excited-state tyrosinate emitting at and around 350 nm was determined after excitation with light of a suitable wavelength, e.g., 275 nm. The experimentally obtained fluorescence decay, which is described as a sum of exponentials, was deconvoluted, and the lifetime of the longest component due to tyrosinate was determined. In solvents of intermediate polarity, such as propylene glycol, isopropanol and the like, or in membrane environments, a lifetime in excess of about 11 nanoseconds for the long lifetime fluorescence of the excited-state tyrosinate is diagnostic of the existence of a particularly stable tyrosinate charge transfer interaction in the subject material.

Example 1 -- Fluorescence Properties of Angiotensin II

Angiotensin II was obtained from Sigma (acetate form) and from Peninsula Labs (trifluoroacetate form) and was found to contain a single peptide by reverse-phase HPLC. Analogs of Angiotensin II were synthesized, purified and bioassayed by method described by Matsoukas et al., J. Med. Chem., 31, pp. 1418-1421 (1988). 1,2-propanediol [$Pr(OH)_2$] was dried by refluxing over calcium oxide for 8 hours, collected by distillation and stored over a molecular sieve. Water content was estimated by ^1H NMR or by the Karl Fisher method. Isopropanol (PrOH) was of HPLC grade (Caledon Laboratories Ltd) and was expected to contain less than 1% water. Dimethyl sulfoxide (DMSO) and trifluoroethanol (TFE) were used without further treatment. Aqueous solvents were prepared from distilled water which had been passed through Fish r i n-exchange cartridges. N-acetyl-

tyrosine-amide (NAYA) was obtained from Sigma. Sodium dodecylsulfate (SDS) was obtained from BDH biochemicals (specially pure) and was used without further treatment.

Fluorescence experiments were performed at 21°C and sample concentration of Angiotensin II used for fluorescence analysis were typically between 0.25 and 1.0 mg/mL. The samples can be warmed to about 50°C to facilitate dissolvement of the peptide. If desired, the samples can then be filtered. Cuvettes were cleaned with sulfochromic acid and were soaked in the highly purified solvent of the experiment. Nanosecond time-resolved fluorescence decays were measured at 21°C using Photochemical Research Associates (PRA) fluorescence lifetime instrumentation (System 3000). This instrument utilizes the time correlated single photon counting technique. A PRA 510 flash lamp was utilized as the light source and was operated at 18.6 kHz, with 5.8 kV applied across a 4mm electrode gap under -44 kPa of H_2. The excitation and emission wavelengths were selected using Jobin Yvon monochromators with slits giving an 8nm bandpass. The lamp decay profile was obtained by measuring the scattering of light by a suspension of 2.02μm polyvinyltoluene latex spheres in glycerol/water (1:1) with the excitation and emission monochromators set at the emission wavelength of the sample. In all experiments data were collected until 2.5 x 10^5 photon counts were obtained. Background counts were obtained for each solvent and were subtracted from the sample data; the background obtained during the time of the sample collection was less than 7% of the counts at the tail end of the sample d cay. The bs rv d decay data were

deconvoluted beginning from 5 channels before the channel maximum to the channel which contained the 0.05% of the photon counts present in the channel of maximum counts. The deconvolution method used was that of iterative non-linear least squares. See Grinvald et al., Anal. Biochem., 59, pp. 583-598 (1974). Acceptance of a least squares fit at 95% confidence was evaluated by the reduced chi-squared test, and the quality of fit was evaluated from the residuals, the autocorrelation function of the residuals, and the Durbin-Watson parameter. See Lampert et al., Anal. Chem., 55, pp. 68-73 (1983).

The experimentally obtained fluorescence decay, $f(\lambda,t)$, is described as a sum of exponentials:

$$f(\lambda,t) = \Sigma\, \alpha_i(\lambda) \exp\,[-t/\tau_i(\lambda)] \qquad (1)$$

where $\alpha_i(\lambda)$ and $\tau_i(\lambda)$ are the preexponential weighting factor and fluorescence lifetime of the i^{th} component for a given emission wavelength, respectively. The fraction of the fluorescence intensity that arises from each component is related by:

$$\text{Int } \%(\lambda) = \frac{\alpha_i(\lambda)\tau_i(\lambda)}{\Sigma\alpha_i(\lambda)\tau_i(\lambda)} \times 100 \qquad (2)$$

Normalized fluorescence decay curves for angiotensin II were obtained in propylene glycol, isopropanol and 0.1 M aqueous SDS. Triexponential fits to the data (equations 1 and 2) gave two parameters: the lifetime of the longest fluorescence component (LLF), and the percentage of the intensity arising from the longest decay component % LLF. The observed LLF for angiotensin II in propylene glycol was 20.8 nanoseconds and the perc nt LLF was 19. F r

angiotensin II in isopropanol the LLF was 15.5 nanoseconds and the percent LLF was 79. In trifluoroethanol, angiotensin II gave LLF equals 13.0 nanoseconds and percent LLF equals 19. In aqueous SDS (SDS above the critical micelle concentration), angiotensin II gave LLF equals 13.7 nanoseconds and percent LLF equals 14.

The finding that the addition of SDS above its critical micelle concentration in water induces tyrosinate fluorescence suggests that intramolecular hydrogen bond formation of the tyrosine hydroxyl in Angiotensin II could occur in the presence of a cell membrane but not in its absence. Both the stability of the tyrosinate species (LLF) and the percent conformer providing for the tyrosinate species (% LLF conformer) were significantly increased when the SDS micelles were formed in the presence of Angiotensin II (LLF = 13.7) compared to when preformed SDS micelles were added to a solution containing Angiotensin II (LLF = 7.2). The former represents a situation where Angiotensin II becomes trapped within the hydrophobic interior of the micelles, whereas the latter represents binding of the positively charged Angiotensin II to the negatively charged exterior surface of the micelle. Differences in tyrosinate fluorescence in these embodiments indicate that the tyrosinate species is stabilized even in an extremely non-polar (hydrophobic) environment, i.e., environments having a dielectric constant of about 2. Accordingly, such non-polar environments are receptor simulating environments.

Analogs of angiotensin II with agonist activities less than 1% of th ag nist activity f

angiotensin II in the rat uterus assay had LLF less than 11 nanoseconds in propylene glycol, and analogs with about 10% or less agonist activity had LLFs less than 12 nanoseconds in isopropanol. For example, [Sar^1Ile^8]Angiotensin II gave LLF equals 0 in propylene glycol and LLF equals 6.5 nsec in isopropanol.

Changing the concentration of the sample did not affect the parameters obtained, therefore dimerization or multiple aggregates can be ruled out as possible conformations responsible for the LLF component.

The above data demonstrates that the existence of a charge-transfer interaction involving the tyrosinate residue in a specific ligand can readily be determined by evaluating the LLF of the tyrosine moiety in the ligand.

Example 2 -- Structure Activity Relationship For Angiotensin II

Structure activity relationships were conducted on Angiotensin II by preparing the analogs [Sar^1Ala^6]Angiotensin II and [$Sar^1Phe\text{-}NH_2$]Angiotensin II. The LLFs, % LLF and Agonist Activity for these analogs are set forth in Table I above. The absence of LLFs greater than about 12 nanoseconds in these analogs implicates both the imidazole and C-terminal carboxylate of Angiotensin II in the charge-transfer interaction. Additionally, the lack of significant agonist activity in the analogs corroborates this finding.

Example 3 -- Fluorescence Properties and Structural Activity Properties of Oxytocin

Using the fluorescence technique described above, oxytocin had an LLF equal to 18.5 nanoseconds in propylene gylcol. On the other hand, an analog of oxytocin, [Ala^5], had an LLF of 7.6 nanoseconds in propylene gylcol. Accordingly, this establishes that the asparagine occupying position 5 in oxytocin is involved in the charge-transfer interaction. Additionally, this analog possessed no agonist activity, which corroborates this finding.

NMR SPECTROSCOPY

Conformational analysis of ligands is achieved by 2D COSY coupled with 1D NMR, 1D NOE enhancement or 2D NOE (ROESY) methods, using a receptor simulating solvent. If the receptor simulating solvent does not contain exchangable deuterium groups, then small amounts of D_2O can optionally be added to the DMSO for exchange purposes. NOE or ROE effects observed as a result of intramolecular through-space relaxations are recorded; interresidue NOEs illustrate the proximity of neighboring groups and thereby provide valuable conformational information. This information is used to construct a molecular model of the ligand. The procedure is facilitated if the presence of a tyrosinate-forming interaction has already been established by fluorescence spectroscopy.

Example 4 -- 2D-ROESY PROTON NMR STUDY OF [SAR^1]ANGIOTENSIN II

[Sar^1]Angiotensin II was synthesized by the solid phase techniqu and purified to homogeneity by reversed-phase HPLC using methods described by

Matsoukas et al., J. Med. Chem. 31(7), pp. 1418-1421 (1988). The synthetic peptide gave the required amino acid analysis and appeared as a single product in two thin layer chromatography (TLC) systems. [Sar^1]Angiotensin II had 180% of the bioactivity of Angiotensin II in the rat uterus assay which is also described by Matsoukas et al., supra. Since HPLC afforded the trifluoroacetate salt of the peptide, [Sar^1]Angiotensin II was neutralized by passage through a column (1.5 x 3 cm) of carboxymethylcellulose (Whatman CM23) cation exchange resin. The peptide (10mg) was first applied to the column in 0.01 M ammonium acetate at pH 5 (5 ml) and then eluted with 0.5 M ammonium acetate at pH 8 (10 ml). The effluent obtained at pH 8 was lyophilized thrice and 5 mg of the product was dissolved in 0.5 ml of DMSO-d_6 and two drops of D_2O were added. Argon was bubbled through the sample for 5 minutes before the NMR tube was sealed.

NMR experiments were carried out using a Bruker AM 400 MHz NMR spectrometer, which was modified to perform spin-locking with an effective radio frequency field of 5 KHz at ambient temperature (297 ± 1°K). Data acquisition and data processing were controlled by an Aspect 3000 computer equipped with an array processor using Bruker 1987 DISNMR software. The chemical shifts were reported relative to the undeuterated fraction of the CH_3 group of DMSO-d_6 at 2.50 ppm with respect to TMS. One-dimensional spectra were recorded with a sweep width of 6100 Hz, and 32 K (zero filled to 64 K) data points. A total of 64 scans were accumulated to obtain a good signal-to-noise ratio. Methods used were as described by Otter et al., Biochemistry, 27(10), pp. 3560-3567, (1988), and Marion

et al., Biochem. Biophys. Res. Commun., 113(3), pp. 967-974 (1983). The parameters employed in the two-dimensional NMR techniques are summarized in Table II below:

TABLE II

Summary of Experimental Parameters used in the Two-Dimensional NMR Experiments[e,f]

Parameters	Unit	COSY	ROESY PH
Sweep Width in F_2 (H_2)	Hz	4000	3300
Sweep Width in F_1 (H_2)	Hz	2000	1650
Matrix size (F_1 x F_2) before zero filling	--	512x1K	256x1K
Matrix size (F_1 x F_2) after zero filling	--	1Kx8K	1Kx2K
Evolution time initial value (μs)	μs	3	1
increment (ms)	ms		78
No. of scans (dummay scans)		32	64
Acquistion time	s	0.32	0.20
Relaxation delay (HDO presaturation)	s	1.8	1.8
Other delays[g]	ms		200
Window functions for 2D FT (F_1/F_2)		5/5	5/5
Shifts of window functions in fractions of $\pi(F_1/F_2)$		4/8	3/4

e = all spectra were recorded at 297°K at 10mM concentration in DMSO-d_6 (+ 2 drops D_2O)
f = After the Fourier transformation, the phase, was optimized in both dimensions by an additional phase correction applied to the entire matrix. The ROESY spectrum was baseline corrected in F^1 and F_2 by means of a Bruker ABS baseline correction.
g = Spin locking time, at an average rf field of 5 KHz.

Both pulse sequences incorporated a decoupler presaturation int rval to suppr ss th water signal. The resulting 2D matrices were displayed and slight

phase adjustment in both dimensions were usually necessary to obtain the best possible data representation. Correlations were verified by examining individual rows and columns of unsymmetrized and symmetrized spectra.

The ROESY experiment required a basic 90° phase correction in t_1 before the phase fine tuning could be done. A carrier frequency of 3.7 ppm, a spin-locking time of 0.2 sec. and 30° flip angle for the hard pulse spin locking train were selected. It should be noted that this experiment often suffers from spurious resonances due to magnetization transfer between scalar coupled spins. Under the selected experimental conditions, the resulting two-dimensional spectrum was almost free of such peaks, which are otherwise easily identified by their phase being the same as the diagonal signals (real ROE cross-peaks have opposite signs with respect to the diagonal peaks). Some baseline distortions were present, especially around intense peaks such as the residual solvent signals and methyl groups. To diminish this problem, spectra were treated in both dimensions with the Bruker ABS baseline correction routine using a polynomial fitting of fifth degree to the baseline. Since all the recorded two-dimensional spectra suffer from considerable t_1 noise and ridges, caution was exercised to obtain reliable information from the spectra. Because only coupling connectivities were of interest, we found it useful to record the magnitude spectrum in the case of the COSY experiment; the ridges along t_1 can then be reduced considerably by methods described by Otter et al., supra.

All resonances of the peptide were assigned to individual amino acids by combined information from COSY and ROESY spectra as set forth in Example 5 below. Our assignments for [Sar^1]Angiotensin II differ from those reported previously for Angiotensin II [by Smeby et al., Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Ed. Weinstein, Dekker, New York, pp. 117-162 (1976)] only with regard to the relative positioning of the Phe C_α proton within the C_α group. Symmetrized and unsymmetrized ROESY spectra were examined to identify both intraresidue and interresidue cross-peaks. Table III below shows all ROESY interactions, both intraresidue and interresidue:

TABLE III

Proton:proton ROESY interactions identified for [Sar^1]Angiotensin II in DMSO-d_6 + D_2O[h,i,j,k,l]

	Sar	Arg(R)	Val(V)	Tyr(Y)	Ile(I)	His(H)	Pro(P)	Phe(F)
Intraresidue interactions	α:M	α:β α:β´ β:γ γ:δ α:γ α:δ β:δ	α:β β:γ α:γ	α:β α:β´ β:β´ α:m β:m β´:m m:o	α:β β:γ β:γ´ γ:δ α:γ α:γ´ γ:γ´ α:M β:M	β:β´ α:C_4	α:β α:β´ β:γ´ γ:δ γ´:δ´ δ:δ´	α:β β:β´ α:F_Φ β:F_Φ β´:F_Φ
Interresidue interactions	M:Y_o		V_M:Y_m	Y_m:V_M Y_m:F_Φ Y_o:Sar_M		H_α:P_δ H_α:$P_{\delta'}$	P_δ:H_α $P_{\delta'}$:H_α P_γ:F_Φ	F_Φ:Y_o F_Φ:P_γ

h = M stands for methyl group protons.
i = Meta (m) and ortho (o) refer to the hydroxyl group on tyrosine.
j = The signals for phenylalanine ring protons (Φ) overlap and were not individually assigned.
k = β', γ', and δ' r f r to the upfield geminal proton resonance.

1 = The amino acid abbreviations us d below are the conventional art recognized abbreviations.

Interresidue interactions are extremely important for studying the conformation of [Sar^1]Angiotensin II in DMSO. Thus the cross-peaks for the Tyr ortho:Phe ring protons, together with the cross-peaks for Pro C_γ:Phe ring protons and Pro C_δ:His C_α protons, illustrate the proximity of Tyr with both His and Phe and suggest that the three aromatic rings in Angiotensin II are in close proximity.

Useful information concerning the rotational freedom of the three aromatic side chains was obtained by examining the intraresidue ROE connectivities between C_α and C_β protons. For Tyr the interaction of the C_α proton with the C_β proton (δ=2.80 ppm) was much stronger than the interaction of the C_α proton with the C_β, proton (δ=2.62 ppm), indicating hindered rotation of the Tyr side chain. For Phe only the C_β, proton (i.e., the proton at δ=2.85 ppm) interacts with the C_α proton (δ=4.10) again indicating the presence of a preferred rotameter and possibly less motion for the Phe side-chain than the Tyr side-chain. For His neither of the C_β protons (δ_1=2.86 ppm and δ_2=2.75 ppm) appeared to interact with the C_α proton (δ=4.60 ppm). By examining rows and columns only a very weak interaction could be observed between His C_α and C_β protons; this suggests that the C_α-C_β bond of His may be essentially fixed in the <u>trans</u> form in DMSO.

The interaction of the His C_α proton with both Pro C_δ protons not only illustrates that the His^6-Pro^7 bond exists primarily in the <u>trans</u> f rm, but also

defines the orientation of the His-Pro bond. A further connectivity present in the ROESY spectrum involves a strong interaction between a methyl group of Val or Ile with the C_α proton of Ile, Val or Phe. Definitive assignment of these cross-peaks could not be made because of signal overlap, but probably represent an intraresidue interaction of Ile and/or Val. Similarly, an interresidue interaction between Tyr meta and a methyl group of Ile or Val could not definitely be assigned. A weak connectivity was also observed between the Sar NCH_3 proton (δ=2.23 ppm) and a Tyr ortho proton (δ=6.58 ppm). A connectivity between the Tyr ortho and His C_4 ring protons was observed in rows but not in columns.

For Arg, the C_α proton (δ=4.32 ppm) appeared to interact with all side-chain methylene protons. Thus, connectivities were observed between the C_α proton and 1) the two C_β protons (1.65 and 1.35 ppm), 2) the two C_γ protons (1.48 and 1.42 ppm), and 3) a C_δ proton at 3.02 ppm (another connectivity between the Arg C_α proton and a proton at 3.45 ppm was tentatively assigned to a Arg C_δ proton). Non-equivalence of the three geminal proton pairs may indicate restricted rotation for the Arg side-chain. ROEs between C_α and C_δ protons may illustrate folding of the Arg side-chain.

Interactions between the Tyr ortho:Phe ring protons, together with connectivities between the Phe ring:Pro C_γ protons and His C_α:Pro C_δ protons, suggest that all three aromatic rings in Angiotensin II are in cl se proximity and form a cluster in DMSO. No ROE cross-p aks were observed between the His and Phe

residues. A previous reported describing shielding of the His ring by the Phe ring in DMSO [see Matsoukas et al., Biochem. Biophys. Res. Commun., 122(1), pp. 434-438 (1984)] may reflect indirect effects, resulting from clustering of the three rings. Alternatively, the His and Phe rings may be separated by a distance which allows for an electrostatic interaction but is beyond the maximum range (<5 Angstroms) for an observable ROE. Similar considerations may explain the absence of an observable two-way ROE between the Tyr and His rings.

The relative orientation of the rings to one another in the cluster cannot be deduced without further information. However, some helpful information is supplied in the form of connectivities between non-aromatic protons, as illustrated in Table III. In particular, the observation of through space interactions between the His C_α and the two Pro C_δ protons defines the orientation of the His-Pro backbone and demonstrates the predominace of the trans isomer in DMSO. It can be deduced that the C-terminal Phe residue must swing around through about 90° in order that the Phe ring can interact with the central Tyr ring (Table III). Modelling experiments illustrate that the Phe residue is most likely to approach the Tyr-Ile-His sequence from a γ turn originating at the His-Pro bond.

The ROESY spectrum shows strong intraresidue $C_\beta/C_{\beta'}$ interactions in all three aromatic residues (Tyr, His, and Phe), illustrating non-equivalence and restricted rotation for these geminal protons. Moreover, non-equivalent interresidue interactions between the C_α proton and the two C_β protons were

observed for Tyr, only one C_α/C_β intraresidue interaction was observed for Phe, and C_α/C_β interactions for His were very weak. These finding suggest that the C_α-C_β bonds of all three aromatic side-chains are unable to freely rotate. For His the C_α-C_β bond protons appear to be locked in the trans position, whereas for Tyr and Phe the C_α-C_β bond protons may be fixed (on the NMR time scale) between the gauche and trans orientatins. Restricted rotation for the Phe ring may originate from interaction with the His side chain, the C-terminal carboxylate and/or an interaction with the Pro ring. The latter interaction is evidenced in the ROESY spectrum by cross-peak connectivities between Phe ring and Pro C_γ and C_γ, protons. These interresidue interactions were non-equivalent; the interaction of the Phe ring proton(s) with the lower field Pro C_γ proton at δ=1.70 ppm, appeared to be considerably stronger than the interaction with the upfield Pro C_γ proton at δ=1.50 ppm. This indicates that the Phe and Pro rings are close but that the Phe ring probably approaches the Pro ring in a non-parallel manner. These findings are in agreement with previous proposals which have suggested functional roles for the three aromatic side-chains, and steric/spatial roles for Ile and Val. See Moore, Pharmacol. Ther., 33(2-3), pp. 349-381 (1987). More specifically, it has been suggested that motion of the His and Phe side-chains would be inhibited by interaction with the C-terminal carboxylate and each other, whereas the Tyr side-chain would be constrained by H-bonding with His. Such considerations could explain why the C_α-C_β bond of His exists predominantly in the energetically less favorable eclipsed conf rmation, since th energy gain d from the charg -

transer interaction could overcome the energy loss due to an eclipsed His C_α-C_β rotomer.

According to the overall structural features suggested by the present ROESY experiment, certain previously proposed interactions [see Moore et al., Biosci. Rep., 5(5), pp. 407-416, (1985)] are permissable. Thus, modelling experiments illustrate that the Tyr hydroxyl could hydrogen bond to an imidazole ring nitrogen of the His residue and that the C-terminal carboxylate could also interact with the His ring. These interactions appear to be possible in DMSO without introducing undue constraint in the molecule. Proximity of the C-terminal carboxylate to one of the His ring nitrogens would serve to increase the polarization of the His ring dipole, thereby increasing the basicity of the other His ring nitrogen and the strength of its interaction with the Tyr OH. This interaction may result in the production of a tyrosinate species which activates the receptor.

The present investigations have shown an interresidue interaction of the Sar^1 residue with the Tyr^4 ring. [Sar^1]Angiotensin was selected in part for this study so that information on interactions of the N-terminal could be readily observed. In this regard, 1D NMR spectra conducted for Angiotensin II and [Sar^1]Angiotensin II in DMSO have shown that the aromatic region of these spectra are identical. The importance of the Sar^1 residue in contributing to the antagonist activity in Sarmesin is well documented and previous studies have shown that the N-CH_3 of Sar^1 is subjected to a shielding influence in a number of angiotensin analogu s in DMSO. See Moore et al.,

Biosci. R p., 5(5), pp. 407-416, (1985). The results of this ROESY example support previous suggestions [Matsoukas et al., Peptides 1986, Ed. Theodoropoulos et al., Berlin, New York, pp. 335-339 (1987)] that the Sar NCH_3 interacts with the Tyr ring. In particular, examination of the rows and columns containing the Sar NCH_3 of the symmetrized and unsymmetrized ROESY spectrum indicated a weak connectivity with a Tyr ortho proton. Thus the source of the shielding effect on the NCH_3 could be the Tyr ring, and the Sar NCH_3 group may exist just at the limiting range for observing an ROE.

Summarizing the above ROESY results, cross-peaks between aromatic rings provided evidence that the three aromatic rings of [Sar^1]Angiotensin II cluster together. Cross-peaks between the His C_α proton with both Pro C_δ protons illustrated that the His^6-Pro^7 bond exists primarily in the trans form. Cross-peaks between the Sar NCH_3 proton and a Pro ortho proton illustrated proximity of the N-terminus of the peptide with the Tyr ring. An observed cross-peak between the Phe ring protons and the Pro C_γ protons illustrated that the Phe ring is close to the Pro ring [as well as the His ring, previously noted].

The information obtained from the 2-D ROESY experiment together with the fluorescence data showing the presence of a tyrosinate charge transfer system, enables the construction of a molecular model for [Sar^1] angiotensin II. This model is shown in FIGURE 1.

Likewise, by following the procedur s s t forth above and because similar cross-peaks have been

observed for angiotensin II, a molecular model of Angiotensin II has been constructed and is set forth in FIGURE 4A. Stereo photographs of this model are illustrated in FIGURE 6.

The model for Angiotensin II shown in FIGURE 4A differs from previously reported conformations. In particular, the N-terminus and the Phe ring have been repositioned in FIGURE 4 in order to accomodate the presently observed proximity of the Tyr ring with both the N-terminus and the Phe ring. Repositioning of the Phe ring is also compatible with ab initio calculations of ring pairing interactions which have suggested a perpendicular-plate interaction for the His and Phe rings. Fowler et al., Biochem. Biophys. Res. Commun., 153(3), pp. 1296-1300 (1988).

Example 5 -- 1D-NOE Enhancement For Sarmesin

Sarmesin and [Des^1]Sarmesin were synthesized by in a similar solid phase technique described in Example 4 above. Purification by reversed-phase HPLC afforded the trifluoroacetate salt of the peptides which were neutralized by passage through a carboxymethylcellulose column as described as well in Example 4 above.

NMR experiments were carried out using a Bruker 400 MHz NMR spectrometer. 5 mg of peptide was dissolved in 0.5 ml of DMSO-d_6 and two drops of D_2O were added. Argon was bubbled through the sample for 5 min. before the NMR tube was sealed. Data acquisition and data processing were controlled by an Asp ct 3000 computer equipp d with an array processor using 1987 DISNMR software. Th chemical shifts wer

reported relative to the undeuterated fraction of the methyl group of DMSO-d_6 at 2.50 ppm with respect to TMS. One-dimensional spectra were recorded with a sweep width of 4500 Hz, and 32 K (zero filled to 64 K) data points. A total of 64 scans were accumulated to obtain a good signal-to-noise ratio. The methods used were similar to those reported by Otter et al., J. Am. Chem. Soc., 109, pp. 6995-7001 (1987). The COSY (two-dimensional correlated spectroscopy) experiments provided contour plots which were symmetrized with respect to the diagonal. The nonselective longitudinal ^1H relaxation times were determined in DMSO-d_6+D_2O (2 drops) using a 180-τ-90° plus sequence and are presented in Table IV below.

TABLE IV
Proton T_1 Relaxation Times (in seconds) for Sarmesin

A. Aromatic Side Chains

His	Tyr(Me)
C_2H 0.332	meta 1.180
C_4H 0.481	ortho 1.505
	CH_3 0.994

B. Backbone protons

His	Tyr(Me)	Ile	Val	Phe(Arg,Pro)
H_α 0.436	H_α 0.962	H_α 0.726	H_α 0.853	H_α 0.896

C. Other

Sar	
H_α 0.559	CH_3 0.805

Several τ values ranging between 0.01 and 10s were employed. Relaxation delays of up to 10s were used for T_1 measurements.

One-dimensional NOE enhancement measurements were carried out in the difference mode using multiple irradiation. Each of the selected lines was irradiated 50 times for 100 ms (total irradiation time 5.0s). Other irradiation times (0.2, 0.5, 1 and 3s) were also employed in some experiments to monitor the NOE build-up. The multiple irradiation procedure allows a very low decoupler power setting (typically 10 dB lower than for a standard NOE experiment) so that it is possible to avoid partial saturation of resonances in close proximity. A total of 1000 scans for each line was required, and total relaxation time was 2s. Under the experimental conditions used for the NOE experiments (low power, different τ preirradiation times, saturation of control areas), spin diffusion and partial saturation were visibly minimized for the interactions under discussion. NOE enhancements were determined as the point increase in signal size per proton after saturation of a functionally distinct proton. Table V below sets forth the resulting NOE enhancements:

TABLE V

NOE enhancements for Sarmesin and [Des[1]]Sarmesin[m]

Peptide	Proton(s) sat.	Enhancement	% proton	Rat.
A	Tyr(Me) C_α	Tyr(Me) $C_{\beta\beta'}$	8.7	C
A	Phe C_α [+Val, Ile]	Phe $C_{\beta\beta'}$	2.4	C
A	His C_α	His $C_{\beta\beta'}$	8.2	C
A	His C_α	Pro C_δ	11.3	D
A	His C_α	Pro $C_{\delta'}$	8.6	D
A	Arg C_α	Arg $C_{\beta\beta'}$	2.5	C
A	Arg C_α	Arg $C_{\gamma\gamma'}$	1.5	C
A	Arg C_α	Arg $C_{\delta\delta'}$	1.1	C
A	Sar C_α [+Pro C_δ]	Sar CH_3	1.2	C
A	Sar CH_3	Sar C_α	0.9	C
A	Sar CH_3	His C_β	1.1	D
A	Pro C_δ [+Sar C_α]	His C_α	5.0	D
A	Pro C_δ [+Sar C_α]	Pro $C_{\delta'}$	9.0	C
B	Tyr(Me) CH_3	His C_2	0.4	D
B	Tyr(Me) CH_3	His C_4	0.5	D
B	Tyr(Me) CH_3	Tyr(Me) ortho	6.7	C
B	Tyr(Me) CH_3	Tyr(Me) meta	3.2	E
B	Tyr(Me) meta [+Phe]	Tyr(Me) ortho	18	C
B	His C_2	Tyr(Me) meta	2.1	D
B	His C_2	Tyr(Me) ortho	1.6	D

m = in DMSO-d_6

A = Sarmesin
B = [Des[1]]Sarmesin
C = intraresidue NOE
D = interresidue NOE
E = spin diffusion

Sarmesin and [Des[1]] Sarmesin were subjected to two-dimensional correlated spectroscopy (COSY) and nuclear Overhauser enhancement (NOE) experiments, suitable for resonance assignment and distance information. It was possible to assign peptide resonances to individual amino acids by combining information from the COSY and NOE difference spectra. The one-dimensional NMR sp ctra of Sarmesin and [Des[1]]

Sarmesin in DMSO-d_6 showed a complex downfield region with broad overlapping NH resonances indicating fast exchange. To simplify the C_α proton and aromatic regions and to study the intramolecular proton-proton interactions between aromatic rings and interresidue backbone protons, the NMR experiments were carried out after the NH's were exchanged with D_2O.

The NOE experiments combined with the COSY spectrum permit complete assignment of all backbone and side-chain proton resonances. Saturation of the C_α protons of His, Tyr(Me), and Phe (overlapped with the C_α protons of Val and Ile), resulted in an enhancement of the vicinal β protons revealing their pattern and exact position in the crowded aliphatic region of the reference spectrum. Thus, the NOE difference spectrum which resulted after saturation of the His C_α proton at δ=4.64 ppm, showed an AB quartet at δ=2.86 ppm attributable to the two vicinal His C_β protons. This interaction was measured to be 8.2%. Similarly, the NOE difference spectra which resulted after saturation of the Tyr(Me) C_α proton at δ=4.48 ppm and the Phe C_α at δ=4.11 ppm, show an AB quartet for the vicinal Tyr(Me) and Phe C_β protons at δ=2.77 ppm (8.7%) and 2.95 ppm (2.4%).

The NOE difference spectrum resulting after saturation of the His C_α proton also shows two strong resonances at δ=3.20 ppm (11.3%) and 3.49 ppm (8.6%) due to enhancement of the two Pro C_δ protons. Both Pro C_δ protons are almost equally affected, revealing close proximity and equidistance of both of these protons from the His C_α proton. This interaction, which also has been bserv d for [Sar^1]Angiot nsin II in DMSO

(Example 4 above), confirms the presence of the trans form of the His^6-Pro^7 bond and permits insight into the relative orientation of the His-Pro-Phe sequence. The stereochemistry around the His-Pro bond in Angiotensin II has been the subject of many investigations using mostly ^1H and ^{13}C-NMR spectroscopy. The results of this example using proton:proton NOE enhancement confirm these findings and, furthermore, define the precise orientation of the His-Pro bond.

Saturation of the C_α proton of the Arg and Pro at δ=4.34 ppm and at δ=4.21 ppm, respectively, revealed multiplet patterns for the respective vicinal ß protons at δ=1.58 ppm and δ=1.75 ppm in the NOE difference spectra. A resonance enhancement observed at δ=3.02 ppm (1.6%) upon saturation of the Arg C_α proton was tentatively attributed to an interaction with the Arg C_δ protons. This interaction, observed also in [Sar^1]Angiotensin II during rotating frame nuclear Overhauser effect spectroscopy studies (Example 4), illustrates proximity of the Arg C_α protons with the Arg C_δ protons. Saturation of the Sar C_α protons resonance at δ=3.2 ppm resulted as expected in the enchancement of the Sar CH_3 proton resonance at δ=2.26 ppm (1.2%). Conversely, irradiation of Sar CH_3 enhanced the Sar C_α protons (0.9%). In addition, enhancement of one of the His C_β protons was observed after Sar NCH_3 saturation, implying proximity of the Sar CH_3 protons to one of the His C_β protons. The observed enhancement of the His C_α proton resonance at δ=4.64 ppm (5%) and the downfield Pro C_δ proton resonance at δ=3.48 ppm (9%) can be attributed to the saturation of the upfield Pro C_δ proton resonance which

is overlapped with the Sar C_α proton resonance at δ=3.20 ppm.

To investigate the proposed proximity of the Tyr and His rings, [Sar^1]Angiotensin II and [Des^1]Angiotensin II (Angiotensin III) were methylated at the Tyr hydroxyl so as to provide a suitable probe (δ - 3.61 ppm) for investigating interaction between the two aromatic rings. For these reasons, NOE experiments were carried out by saturating the Tyr OCH_3 resonance and the His C_2 and C_4 proton resonances in both analogs. Upon saturation of the Tyr OCH_3 resonance of [Des^1]Sarmesin ([Tyr(Me)^4]Angiotensin III), weak enhancements of the His C_2 and C_4 proton resonances at δ=7.47 ppm (0.42%) and δ=6.85 ppm (0.52%), respectively, were observed. The weakness of these interactions places the Tyr OCH_3 group at the limit of the permissable distance (< 5 Angstroms) from the His imidazole ring for the effect to be observed. The cancellation of the Phe ring resonance at the vicinity of the Tyr meta resonance suggests that the observed effect may be real and not due to spin diffusion. However, enhancement of the Tyr ortho and meta proton resonances at δ=7.82 ppm (3.21%) and δ=6.71 ppm (8.86%) was observed upon saturation of the Tyr OCH_3 protons in [Des^1]Sarmesin. Whereas the former is an expected Overhauser effect, the latter may result from second-order magnetization transfer via the ortho protons. The possibility cannot be ruled out that the enhancements of His C_2 and C_4 in [Des^1]Sarmesin result from secondary NOEs relayed by Tyr ortho and/or meta protons, although such considerations would still place the Tyr and His rings in close proximity. Since similar NOE enhancements of the His C_2 and C_4 prot ns

were not observed for Sarmesin, the presence of the N-terminal Sar may subtlely alter the conformation of the octapeptide and place the Tyr OCH_3 group just outside the boundary for permissible and observable NOE interactions with the His ring.

Saturation of the His C_2 proton resonance at δ=7.47 ppm in [Des^1]Sarmesin, resulted in enhancement of the Tyr meta and ortho proton resonances at δ=7.08 ppm (2.15%) and δ=6.71 ppm (1.58%). However, upon saturation of the Tyr meta proton resonance at δ=7.09 ppm (overlapped with the Phe ring proton resonances), no enhancement was observed for the His C_2 and C_4 protons at δ=7.47 ppm and δ=6.85 ppm. Only the Tyr ortho proton at δ=6.71 ppm (18%) in the aromatic region under scrunity was enchanced. The latter saturation serves as a control experiment to show the minimum contribution of partial saturation to the enhancement of the Tyr meta and ortho proton signals.

The NOE difference spectra for Sarmesin upon saturation of the His C_α, Tyr(Me) C_α and Phe (Val, Pro) C_α protons reveals intraresidue C_α/C_β proton interactions in His, Tyr(Me), and Phe. The presence of an interresidue His C_α/Pro C_δ proton:proton NOE defines a predominantly <u>trans</u> conformation for the His-Pro peptide bond of Sarmesin in DMSO. The <u>trans</u> isomer also predominates in [Sar^1]Angiotensin II (Example 4), illustrating that both agonist and antagonist maintain this conformational property in DMSO. Furthermore, the similar effects of saturation of the His C_α proton on both Pro C_δ protons locates the His C_α proton midway between the two Pro C_δ protons.

An interresidue proton:proton NOE was observed between the Arg C_α Arg C_δ protons after saturation of the former in Sarmesin. This could illustrate that the Arg side-chain does not exhibit complete freedom of motion in DMSO, or that it exhibits sufficient conformational freedom to sample many conformations. The role of this positively charged side-chain may be to contact a complementary anionic site on the receptor and assist in bringing about productive binding of Angiotensin II to its receptor. The same interaction has been observed in [Sar^1]Angiotensin II (Example 4). This, together with considerations for the His-Pro bond in [Sar^1]Angiotensin II and Sarmesin, illustrates similarities in certain aspects of the conformations of the agonist and the antagonist in DMSO.

Proximity of the Sar CH_3 protons with one of the His C_β protons in Sarmesin is evidenced by signal enhancement of the latter with saturation of the former. This suggests the presence of a bend in the N-terminal Sar-Arg-Val region of the molecule which allows proximity of Sar and His. Modelling experiments suggest that, for steric reasons, the N- and C- termini of the molecules probably approach the central domain from different sides, thereby creating an approximately S-shaped peptide backbone.

The NOE difference spectrum for [Des^1]Sarmesin upon saturation of the Tyr(Me) methyl protons illustrates enhancement of the His C_2 and C_4 protons. Proximity of the Tyr(Me) methyl group and the His ring is in accord with the previously postulated hydrog n bonding interaction between the Tyr OH and the

His ring in Angiotensin II. Spin diffusion not withstanding, the similar NOE enhancements of both the His C_2 and C_4 protons suggests that the Tyr(Me) ring may have a perpendicular orientation relative to the imidazole ring; this deduction is based on the expected planarity of the methoxy group with the Tyr ring. This is a potentially interesting observation since the relative orientations of the Tyr and His rings may be similarly maintained in the cluster of aromatic rings in Angiotensin II. Even if enhancement of the His C_2 and C_4 resonances is due to secondary NOEs relayed by the Tyr ortho and/or meta protons, the data still establish the important fact that the Tyr and His rings are in close proximity.

Upon saturation of the His C_2 proton in [Des^1]Sarmesin, NOE enhancement of the Tyr(Me) meta and ortho protons is observed. Due to overlap of the His C_4 and Tyr(Me) meta and ortho proton signals in the NMR spectrum, it was not possible to saturate the His C_4 proton and obtain meaningful results. Indeed, the validity of the His C_2 saturation experiment is questionable and a control experiment was carried out to test the extent of partial saturation. Saturation of the Tyr(Me) meta protons resonance at δ=7.09 ppm (overlapped with the Phe ring protons resonance) resulted in enhancement of the Tyr(Me) ortho protons at δ=6.72 ppm (30%) but not of the His C_2 and C_4 protons resonances. This experiment favors the absence of partial saturation effects contributing to the enhancement of the Tyr(Me) meta and ortho protons resonances after saturation of the His C_2 proton, validating the experimental data showing proximity f th Tyr(M) and His rings of [Des^1]Sarm sin in DMS .

Moreover, the interaction between the Tyr(Me) and His rings in [Des^1]Sarmesin is not a reverse relaxation phenomenon. Thus, while the His C_2 and C_4 protons can relax through the closely spaced Tyr(Me) meta and ortho ring protons, the reverse effect is not observed upon saturation of the Tyr(Me) meta and ortho protons. The probable reason for this is that Tyr(Me) ortho and meta protons have relaxation pathways which are not available to the His C_2 and C_4 protons. The Tyr(Me) ortho protons can relax through the Tyr(Me) meta and methyl protons, while the Tyr(Me) meta protons can relax through the Tyr(Me) ortho and C_β protons.

In conclusion, the findings of this example suggest that Sarmesin and [Des^1]Sarmesin contain the same bend at the His-Pro bond which has been observed for [Sar^1]Angiotensin II and that this produces similar clustering of the aromatic rings. Sarmesin and Angiotensin II appear to assume an approximately S-shaped conformation in DMSO. Previous work has suggested that the N-terminus of [Sar^1]Angiotensin II interacts with the Tyr ring, whereas the present findings indicates that the N-terminus of Sarmesin is close to the His side-chain. From molecular modelling experiments, it can be shown that the Sar NCH_3 group can occupy a position which is close to both the Tyr ring and the His C_β protons simultaneously.

Example 6 -- NMR Studies on Oxytocin and [Arg^8] Vasopressin

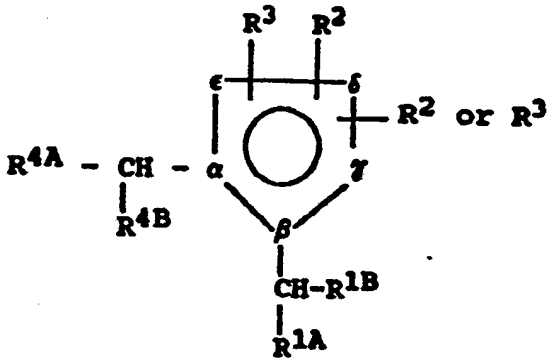
NMR studies were carried out using a Bruker 400 Mz instrument essentially as described in previous examples. Peptides were dissolved at a concentration of 5mg/0.5ml of DMSO-d_6 and 2 drops of D_2O were added.

A characteristic resonance for the tyrosine hydroxyl proton at δ=9.2 ppm was present in the proton NMR spectrum for vasopressin, but not in the NMR spectrum for oxytocin. The absence of this signal in oxytocin is diagnostic for tyrosinate formation and agrees with the fluorescence spectroscopy (Example 3); this signal was also absent for angiotensin II.

A ring pairing interaction for vasopressin is also evident when the NMR data for the Tyr and Phe rings of vasopressin are investigated. Theoretical calculations [Fowler et al., Biochem. Biophys. Res. Commun., 153(3), pp. 1296-1300 (1988)] have illustrated that electrostatic ring pairing interactions will occur preferentially in the perpendicular-plate orientation, and that a slipped parallel-plate configuration will only be adopted when other prevailing factors override perpendicular-plate interactions. In NMR experiments, perpendicular-plate interaction is accompanied by shielding of the protons of one ring and the absence of a shielding effect on the other ring, with both rings demonstrating non-equivalence of their ring protons. This is seen for vasopressin where the Tyr ring protons in the peptide are shielded (6.91 and 6.62 ppm) compared to the protons of free Tyr (7.07 and 6.70 ppm) or the Tyr ring protons of xytocin (7.12 and 6.68 ppm). The Phe ring protons of vasopressin are not

shielded and are non-equivalent (7.34 and 7.24 ppm) compared to free Phe (7.30 ppm). This discloses the fact that the hexagonal axis of the Tyr ring interacts with the hexagonal face of the Phe ring in vasopressin. The method can be used for any molecule where a ring pairing interaction is possible.

Using the charge distribution map depicted in FIGURE 4B, together with the implicit considerations of FIGURES 5 and 7, there are prepared the following new antagonists to the Angiotensin II receptor, based on modifications to the imidazole ring. It should be noted that the nomenclature of the substituents of the ringed compounds is different than that presented above. Thus, the new antagonists to the angiotensin II receptor are compounds of the formula:



wherein $\alpha,\beta,\gamma,\delta$ and ϵ are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom, and (b) attachment of R groups is to C or N, and preferably further with the provisos that (c) at least one ring N atom remains unsubstituted, and (d) the pKa of the ring is $\leq$ 7 when all attendant groups have been taken into account;

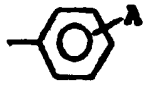
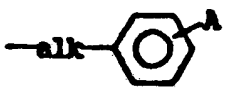
R^{1A}, which mimics the structure in angiotensin of - CH - CO - N - CH - CO -  includes the following:

-alk; -O-alk; -alk-O-alk; $-CH_2-CO-NH_2$; $-CH_2-CO-NH-alk$; $-CH_2-CO-N(alk)_2$; 

$-CH_2-CO-AA-NH_2$; or $CH_2-CO-AA-Phe$,

wherein AA is an amino acid preferably proline, azetidine-carboxylic acid, pipecolic acid, nipecotic acid, glycine, alanine, sarcosine, or N-methyl-alanine;

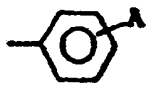
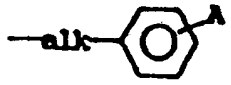
R^{1B}, which optionally provides a spacer arm terminating in a mimic of the C-terminal carboxylate group of angiotensin II, includes the following:

-H; -alk-A;  ; or  ;

preferably with the proviso that when R^{1B} is H, then: (a) if the ring is imidazole α and/or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2;

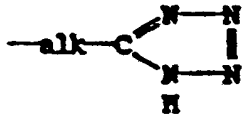
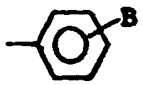
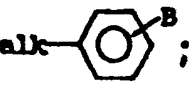
R^2, which provides steric and/or electronic properties and/or a spacer arm terminating in an acid group, includes the following: -H, -halide; -alk;

-O-alk; $-NO_2$; $-CF_3$; -CN; -alk-A; -A;

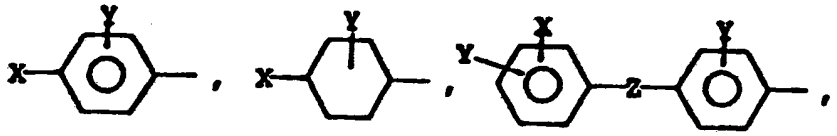

; or


R^3, which provides steric and/or electronic properties and/or a mimetic of the tyrosine hydroxyl group of angiotensin II in its "charge relay" conformation, or a spacer arm terminating in a mimic of the N-terminus of N-terminal dipeptide of angiotensin-II, includes the following;

-H; -alk; -aryl; -alk-OH; -alk-halide; $-CH_2$-O-alk; $-CH_2$-CN; $-CH_2-CO_2H$; $-CH_2CO_2$-alk; -NH-CO-alk; -CO-NH-alk; -alk-B; -CH(OH)-alk-B; -alk-Asp-Arg-NH_2; -CH(OH)-alk-Asp-Arg-NH_2;


;

; or

;

R^{6A}, which provides a spacer arm, the relative rigidity of which is an aspect of the design, terminating in an acid group which mimics the tyrosine hydroxy groups of angiotensin II in its "receptor bound" conformation includes the following: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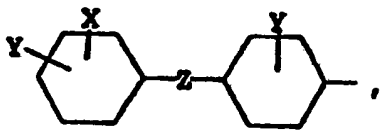
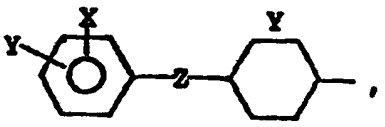

,

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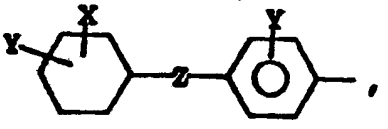
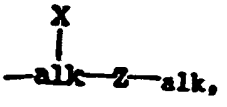
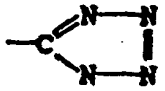
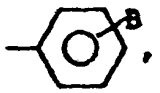
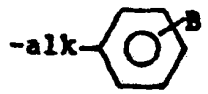

, and

,

where Z is a bond, -NHCO-, -O-, $-OCH_2-$, or $-CH_2-$;
X is $-CO_2H$, -alk-CO_2H, $-PO_3H$, -alk-PO_3H, $-PO_4H_2$, -alk-PO_4H_2, -SH, -alk-SH, $-SO_3H$, -alk-SO_3H, $-SO_4H_2$, -alk-SO_4H_2, $F_3C-CO-NH-$, F_3C-SO_2-NH-,



or yet another acid group
or a pharmaceutically acceptable salt thereof; and
Y is -H, -halide, $-NO_2$, -O-alk, -alk, $-CF_3$, or -CN; and

R^{48}, which optionally provides a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin, includes the following: -H, -alk-B, -alk-Asp-Arg-NH_2, alk-O-alk-B, alk-O-alk-Asp-Arg-NH_2,

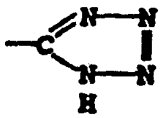

,


preferably with the proviso that when R^{48} is H, then: (a) if the ring is imidazole either α or γ is other N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or is -Asp-Arg-NH_2;

alk = an alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having 3-6 carbon atoms,an alkenyl group having 2-10 carbon atoms, or an alkynyl group having 2-10 carbon atoms;

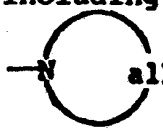
halide = -F, -Cl, -Br, or -I;

A = an acid group or its pharmaceutical salt and includes but is not limited to $-CO_2H$, $-CO_2R^4$, $-CO_2$alk, $-SO_3H$, $-SO_4H_2$, $-PO_3H$, $-PO_4H_2$, $F_3CCONH-$, F_3CSO_2NH-, -alk-SH, or



wherein R' is a lipophilic ester prodrug form such as $-CH_2CO_2C(CH_3)_3$ and the like;

B = a basic group or its pharmaceutical salt including, but not limited to $-NH_2$, -NHalk, $-N(alk)_2$,



In a preferred aspect of the present invention, when R^{1B} is H, then: (a) if the ring is imidazole α and/or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2.

In a further preferred aspect of the present invention, when R^{4B} is H, then: (a) if the ring is imidazole α and/or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2;

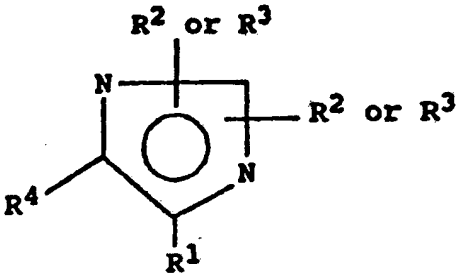
In a particularly preferred product aspect of the present invention, the above five-membered ring is imidazole.

Preferably, there is no duplication of R^2 or R^3 when not equal to H.

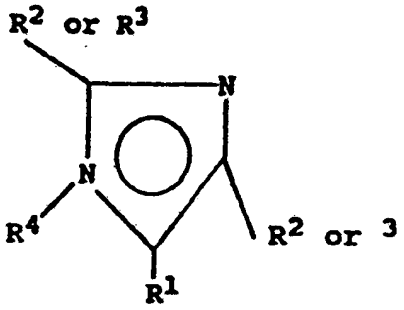
In the above formula, the group $-CH(R^{1A})(R^{1B})$ may be denoted R^1 and the group $--CH(R^{4A})(R^{4B})$ may be denoted R^4. The relative positioning of the R groups set f rth abov , and particularly the relati nship between th R^1 group and th R^4 group, giv s ris to

several different configurations in the case of five-membered rings such as imidazole, pyrole, pyrazole, triazoles, tetrazoles, thiazoles, etc.

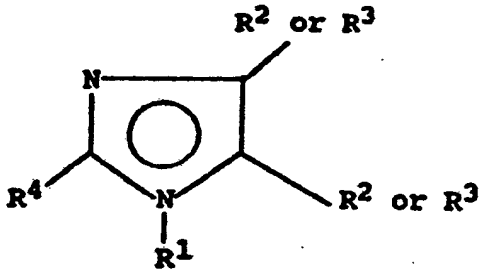
For example, in the case of imidazole, the following configurations apply:



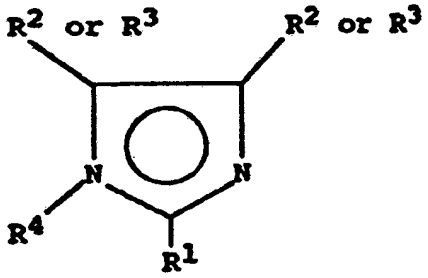
Configuration $N^{\gamma,\epsilon}$



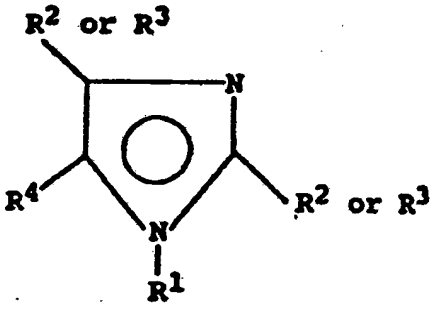
Configuration $N^{\alpha,\delta}$



Configuration $N^{\beta,\epsilon}$



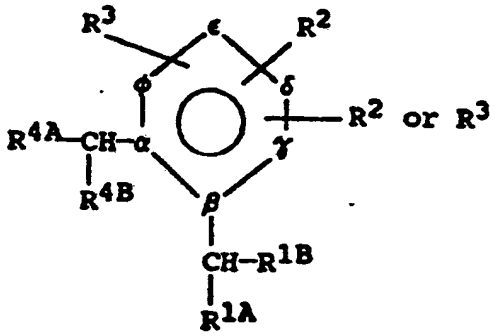
Configuration $N^{\alpha,\gamma}$



Configurati n $N^{\beta,\delta}$

Since R^4 is a substituted benzyl group or equivalent, configurations $N^{\alpha,\delta}$ and $N^{\alpha,\gamma}$ are generically speaking N-benzyl compounds, whereas configurations $N^{\gamma,\epsilon}$ and $N^{\beta,\epsilon}$, and $N^{\beta,\delta}$ are C-benzyl-imidazoles. Similar configurations, numbering ≤5 depending the number of ring N atoms present, apply to other five-membered heterocyclic rings.

The novel antagonists of the present invention are not limited to five-membered rings but indeed encompass six-membered rings including pyridine and diazines (such as, pyrimidine, pyridazine, and pyrazine) as well as triazines. For example, the following may serve as substitute for the imidazole ring in histidine:



wherein α, ß, γ, δ, ε, and φ are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom, (b) attachment of R groups is to C or N, (c) the number of substituted N atoms is one or more, and (d) the pKa of the ring is ≤ 7 when all attendant groups have been taken into account;

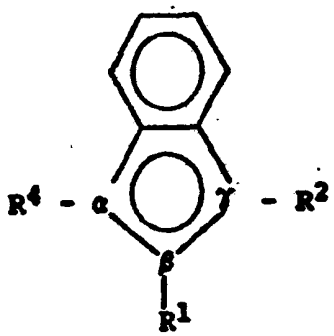
and wherein R^{1A}, R^{1B}, R^2, R^3, R^{4A}, Z, X, and Y, R^{4B}, alk, halide, A, and B are as defined previously.

Preferably, α is other than N.

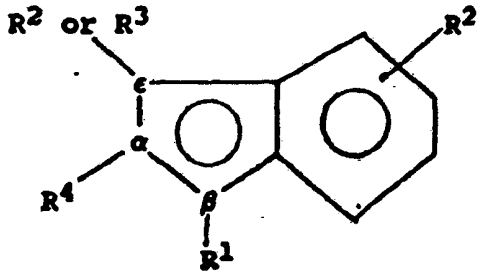
Preferably, ß is N.

Preferably, there is no duplication of R^2 or R^3 when not equal to hydrogen.

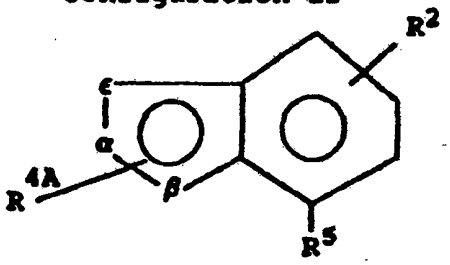
Yet another group of ringed moieties which may be substituted for the imidazole ring of histidine are indoles, benzoazoles, and the like with the further proviso that steric considerations permit that such ring systems fall within the spatial constraints permitted by the conformational models of angiotensin set forth previously. Consideration of these conformational models allows for three general configurations, as follows:



Configuration I



Configuration II



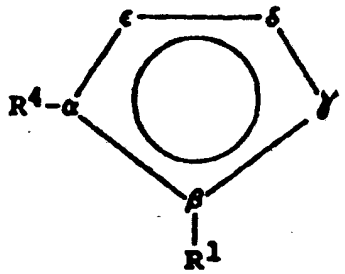
Configuration III

wherein α, ß, and γ are C or N, with the proviso that only one N atom is substituted. R^1 is $-CH(R^{1A})(R^{1B})$ and R^4 is $-CH(R^{4A})(R^{4B})$. Substituents R^{1A}, R^{1B}, R^2, R^3, R^{4A}, R^{4B}

and R^5 are as defined above, except that for these compounds when R^{1B} is H then (a) R^{1A} comprises a group containing an amide, or (b) R^1 is on an N or (c) R^4 is on a C. It is noted that configurations I and II apply to indoles, but not to benzotriazole and benzopyrazole. Configuration I applies to benzimidazole with the proviso that no R^2 group is present at N^7, and configuration II applies to benzimidazole with the proviso that no R^2 or R^3 group is present at N^4. Configuration III applies to indoles, benzimidazole, benzopyrazole and benzotriazole; substitution of R^2 or R^3 at ϵ is optional according to the above provisos provided that only one nitrogen is substituted. R^5=R^1.

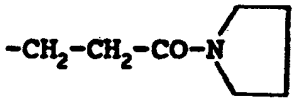
Equivalent considerations apply to six-membered heterocyclic ring systems such as benzopyridine, benzodiazines, purines, quinolines, phenanthrolines and the like.

According to Figure 4 of the parent application and considerations relating thereto, one embodiment of the synthesis of new antagonists is based on incorporating additional charges in appropriate locations in BI and BABI compounds so as to increase the binding affinity of these antagonists to the angiotensin II receptor and accordingly increase their potencies. Such considerations apply not only to N-benzyl- and N-benzamidobenzyl-imidazoles but also to C-benzyl- and C-benzamidobenzyl-imidazoles, as outlined in the parent application and in further detail above for 5 configurations of the imidazole ring:



wherein R^4 = benzyl or benzamidobenzyl which is optionally substituted and wherein α=N or C. According to another consideration inherent to Figure 5 of the parent application, the synthesis of new antagonists also invokes the inclusion of an improved substitutent to replace the n-alkyl group (R^1), namely, a more accurate mimetic of the sequence $-CH_2-\underset{|}{CH}-CO-Pro-$

in angiotensin; such improved mimetics have been outlined above and the following are particularly relevant:

$-CH_2-CH_2-CO-NMe_2$;  ; and
$-CH_2-CH_2-CO-Pro-NH_2$.

In yet another consideration based on Figure 7 and in particular the discussion outlined on pages 43-46 of the parent application, the orientation of the heterocyclic ring is an important aspect of the design of angiotensin antagonists. As outlined previously, five possible orientations or configurations, which are determined by the placement of the N atoms in the ring relative to the R substituents of the imidazole ring, are applicable to the synthesis of angiotensin antagonists. Furthermore, and as outlined previously, ring systems isofunctional with imidazole can also be present in these antagonists, including other azoles, and including six-membered rings such as pyridin , diazines, and the like, as well as polynuclear ring

systems having at least one 5- or 6-membered heterocyclic ring, as described above.

In a preferred embodiment of the present application, the heterocyclic ring is imidazole in any of its five possible configurations outlined previously. A particularly preferred embodiment is configuration $N^{\prime,\epsilon}$ which gives rise to C-benzyl-compounds and which exactly mimics the imidazole group of the histidine residue of angiotensin-II (See figure 5). Furthermore, it is also a preferred embodiment that the R^1 substituent is not a straight chain hydrocarbon but contains an amide function mimicking the His-Pro group in angiotensin II. In yet another preferred embodiment, a spacer arm terminationg in a charged group, which mimics the N- or C- terminus of angiotensin II, is incorporated at R^2 or R^3 or R^1B or R^{4B}, as outlined previously. The criteria for these preferred embodiments are based on molecular modelling of angiotensin as outlined in Figures 4,5, and 7 and relevant discussion thereto, including pages 43-46 of the parent application.

The compounds depicted above can be readily prepared by the skilled artisan using art recognized techniques. Such compounds and their pharmaceutically acceptable salts are useful as Angiotensin II antagonists. Accordingly, such compounds can be used to control hypertension and/or congestive heart failure in a mammal in need of such treatment. Additionally, the compounds of this invention are contemplated as being useful in other cardiovascular and related diseases such as stroke, myocardial infarction and the like. When used to control hypertension and/or

congestive heart failur , the compound is normally administered to such a mammal either orally or parenterally. When so administered, the compound is generally formulated in a pharmaceutically acceptable diluent and at a dosage sufficient to control hypertension and/or congestive heart failure in the mammal so treated. The specific dose levels for such uses can be readily determined by the skilled artisan. Accordingly, the present invention contemplates a method for controlling hypertension in a mammal in need of such treatment which comprises either administering orally or parenterally a pharmaceutical composition of a compound depicted above in an amount sufficient to control hypertension. Additionally, the present invention also contemplates a method for treating congestive heart failure in a mammal in need of such treatment which comprises either administering orally or parenterally a pharmaceutical composition of a compound depicted above in an amount sufficient to control said heart failure. The methods of controlling hypertension are implemented using pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an amount of a compound depicted above effective to control hypertension in a mammal in need of such treatment. The methods of controlling congestive heart are implemented using pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an amount of a compound depicted above effective to control said heart failure.

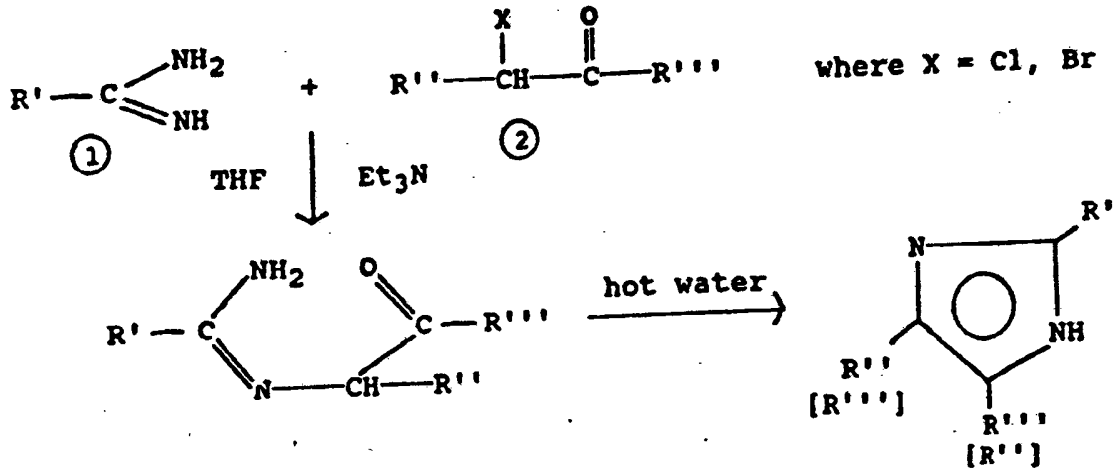
Methods of preparing the above-described compounds are now described. The synthesis of heterocyclic compounds follows methods well known to on skill d in the art, such as methods d scribed in

Comprehensiv H terocyclic Chemistry, P rgamon Press, New York, wherein Vols 4 and 5 (1984) are particularly relevant to the present invention. Synthetic methods of the present invention have also been reviewed in detail in European Patents 0263310 and 0323844. In view of the knowledge in the art concerning the synthetic routes employed, general synthetic schemes for the preparation of compounds according to the invention are presented below.. Such schemes generally utilize combinations of chemical transformations together with strategies and protecting groups familiar to one skilled in the art.

Unless otherwise stated, all reactions are conducted at temperatures ranging from 20°C to the reflux temperature of the solvent for between two hours and two days in a suitably inert solvent such as dimethylformamide, dimethylsulfoxide, chloroform, methylene chloride, benzene, toluene, dioxane, tetrahydrofuran, or ether.

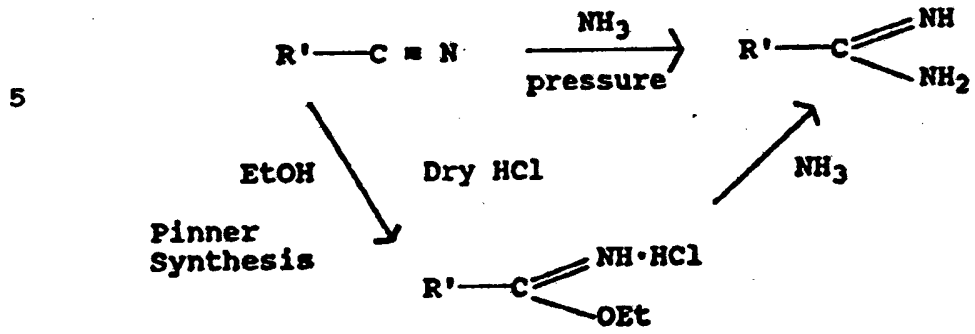
Synthesis of Substituted Imidazoles

The following references are pertinent to the synthesis of substituted imidazoles. Advances in Heterocyclic Chemistry, Vols. 4,12,27,35 (Cambridge University Press) and Heterocyclic Nitrogen Compounds: The Azolesz, K. Schofield et al (1976) Cambridge Univ. Press. A general scheme for the synthesis of substituted imidazoles involves condensation of an amidine or related compound with an α-halo/hydroxy-ketone:



The isomers are separated by conventional methods such as crystallization or chromatography.

The amidine 1 is prepared from the nitrile directly or via the iminoether:



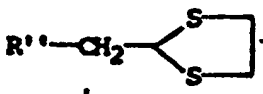
The halo/hydroxy-ketone 2 is prepared by numerous methods known in the art, for example:

(A) $R''-CO_2H$ → (dicyclohexylcarbodiimide, diazomethane/ether) → $R''-C(=O)-CHN_2$

→ (2 equiv. N-chlorosuccinimide dioxane) → $R''-C(=O)-CHCl_2$

↓ $(R)_2CuLi$

$R-CH(Cl)-C(=O)-R''$

(B) $R-CH_2CHO$ → (1,3 propane dithiol) → 

↓ 1) BuLi/THF 2) $R'''I$

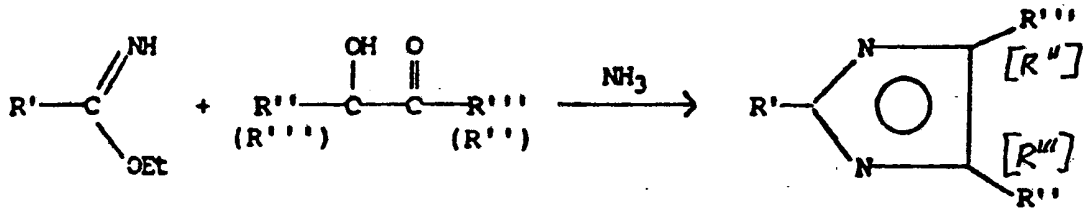
H^+ ↓ 1 equiv. N-chlorosuccinimide

$R-CH(Cl)-C(=O)-R''$

1) BuLi/THF
2) $R'''CHO$
3) Hg^{2+}

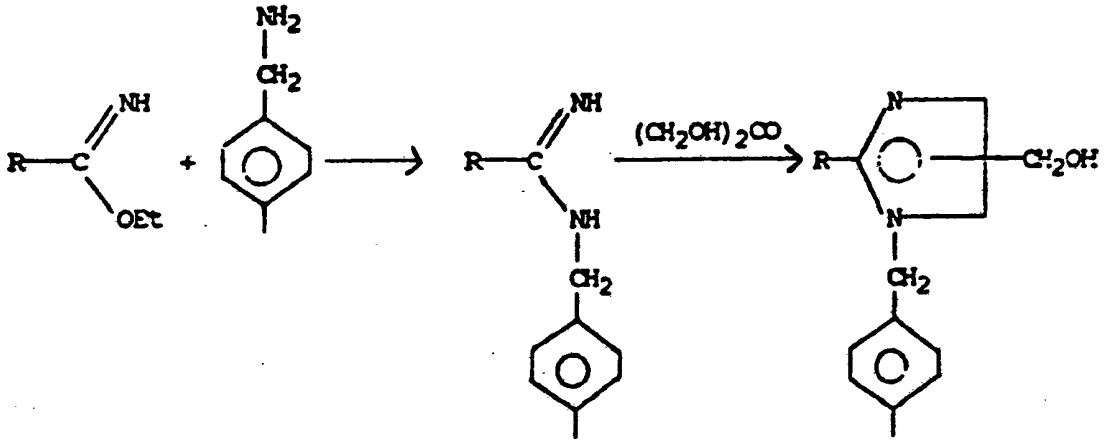
$R''-CH(OH)-C(=O)-CH_2-R$

Alternatively, an iminoether can be condensed with an α-hydroxy/halo-ketone in the presence of ammonia:

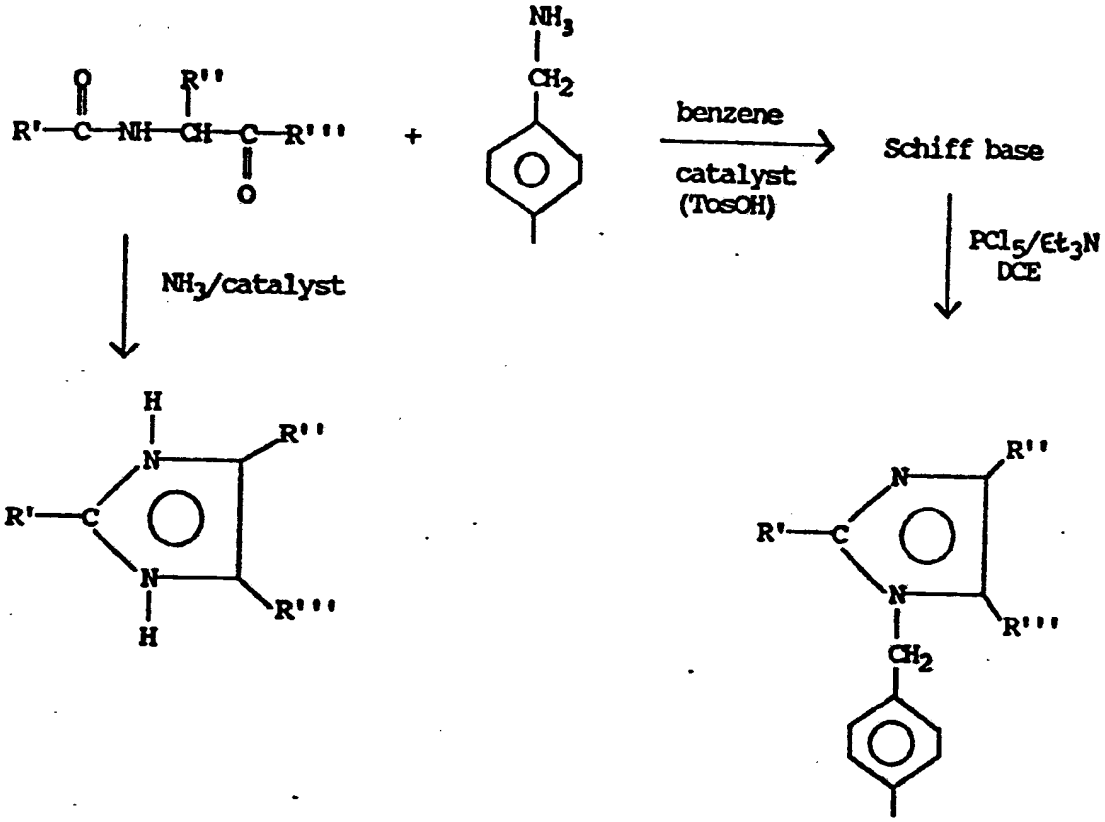


The isomers are separated by crystallization or chromatography.

In another method applicable to substituted N-benzyl compounds in particular, iminoether is reacted with substituted benzylamine to form the amidine which is subsequently condensed with α-halo- or α-hydroxy-ketone/aldehyde:



In yet another method applicable to the synthesis of substituted N-benzyl compounds, acylaminoketone is reacted with derivatized benzylamine to form an imine which is then converted to N-benzylimidazole:

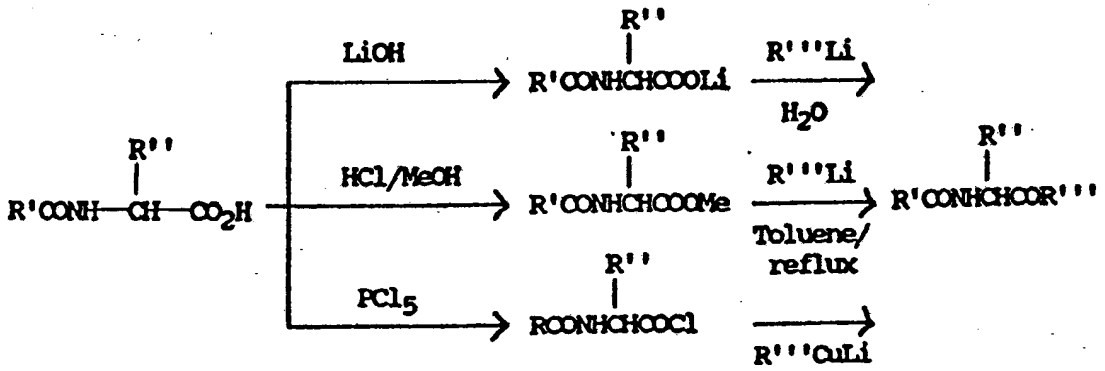


Likewise, imidazole compounds can be obtained using ammonia instead of amine as set forth in Davidson et al, J. Org. Chem, 2, 319, 1937 and Heinze et al, Chem. Ber. 101, 3504, 1968.

Acylaminoketone is readily obtainable from amino acids using the Dakin-West reaction and modifications thereof as well as from the corresponding α-haloketone by art recognized methods.

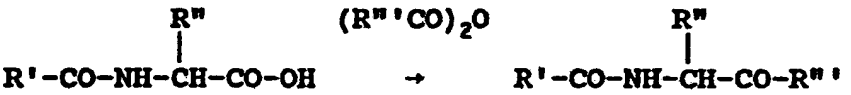
Preparation of Acylaminoketones

Aminoacylketones of the general formula R'-CO-NH-CH(R")-CO-R"' can be prepared frim N-acyl amino acid by reaction with alkyllithium or alkylcopperlithium:



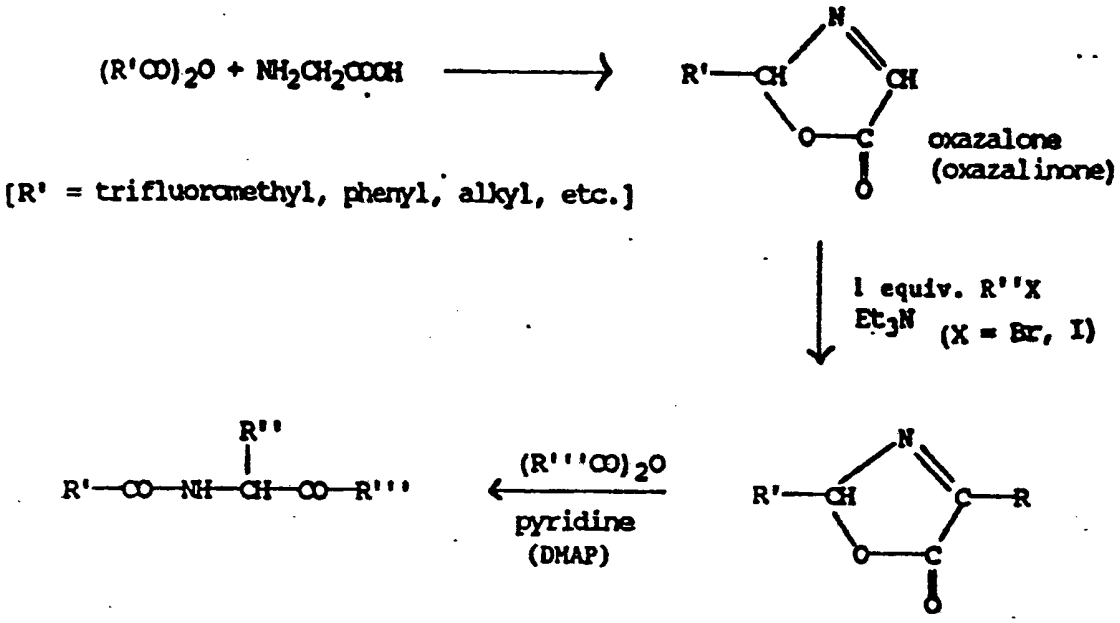
For example, N-acyl-DL-4-nitrophenylalanine can be converted to its butylketone derivative using, e.g., butyllithium.

Another general method for preparing acylaminoketones is by the Dakin-West reaction in which the acyl amino acid is converted to the required acylaminoketone by reaction with anhydride in the presence of base (See Hofle et al, Angew. Chem. Int. Ed. Vol. 17, p. 569, 1978):

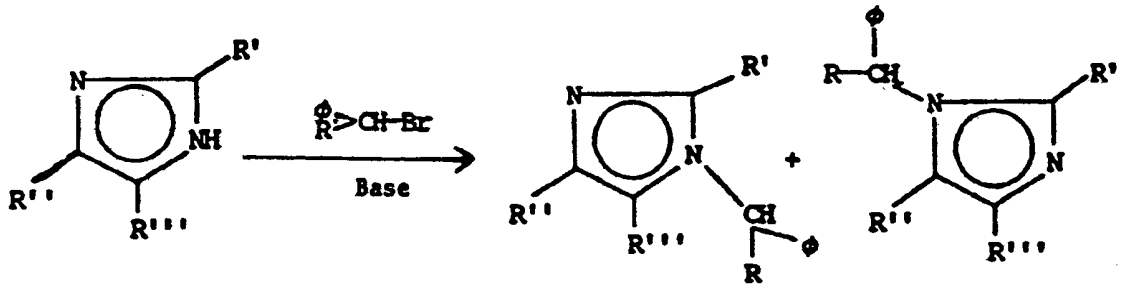


This reaction proceeds through the oxazolinone, and provides an alternative stepwise

approach for preparing acylaminoketones when the amino acid is not readily available. For example, acylglycine can be converted to other acylamino acids by alkylating the oxazalinone intermediate as follows:

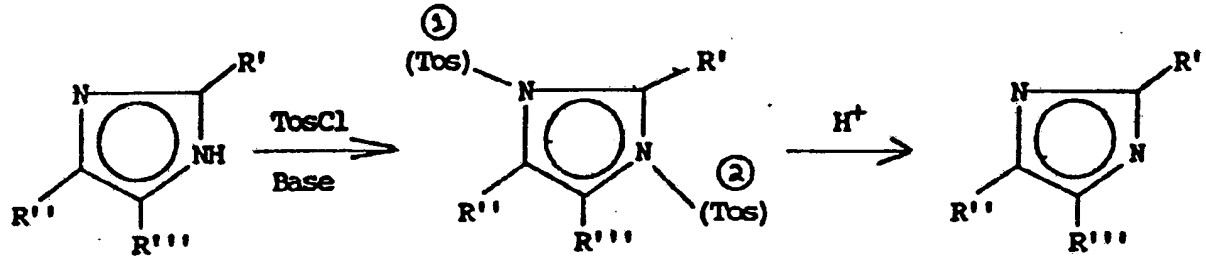


For N-benzyl compounds, alkylation of imidazole nitrogen can be carried out as follows:



The two products can be separated by classical methods such as crystallization and chromatography.

For C-benzyl compounds, the imidazole nitrogen can be protected with a suitable protecting group such as tosyl, benzyloxymethyl, trityl, or benzyl, which can be subsequently removed by a strategically acceptable method such as acidolysis or hydrogenation. If the protecting group is to be removed at the end of the synthesis, the two products formed do not need to be separated:



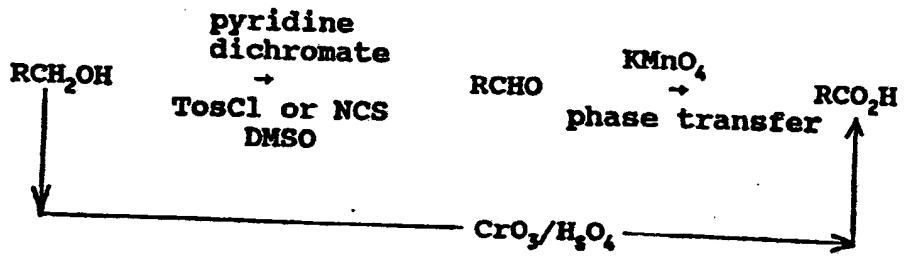
(2 products)

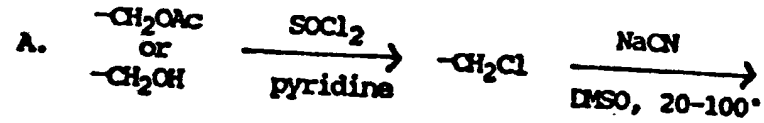
As previously indicated, it is considered to be within the skill of persons in the art to prepare compounds as described. One skilled in the art knows, for example, that it is often not possible to introduce a side group during synthesis in the form which is required in the final product. Thus, for example, an amino group often cannot be introduced in the middle of the synthesis scheme because its reactivity towards electrophiles is high and it could therefore become irreversibly modified during the course of the synthesis to the final product. The amino group is therefore introduced as a nitro group or as an acylamino group or in yet another form in order to circumvent this problem. At or near the end of the synthesis scheme, at which point conversion to an amino group will not compromise the integrity of the final product, th amino gr up is produced using classical chemical procedur s. Similarly, when the end product

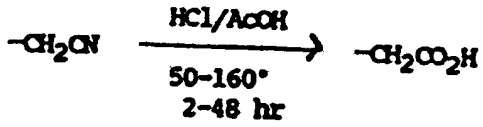
is to contain a carboxylate function, it is introduced in the form of a nitrile, alcohol, ether, ester, alkene or some other art-recognized precursor.

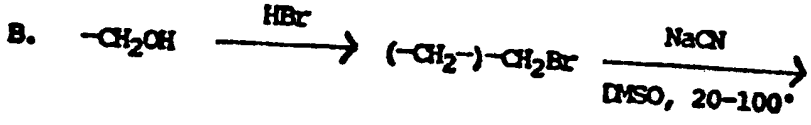
Conversions of this general type are done by the classical reactions shown below, and produce the side-groups indicated. For further details, see J. March, Advanced Organic Chemistry, 1985 J. Wiley & Sons, New York and references therein.

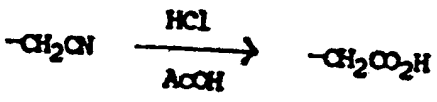
1. Carboxylate:

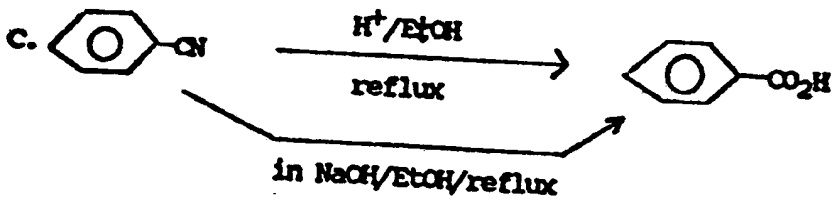




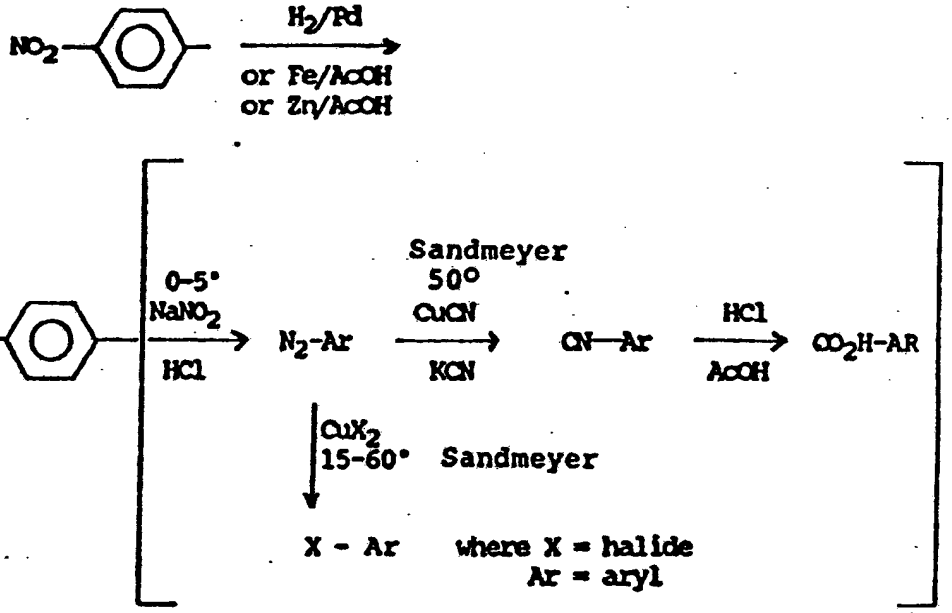








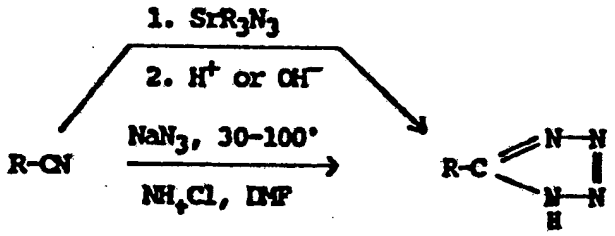
2. Amino



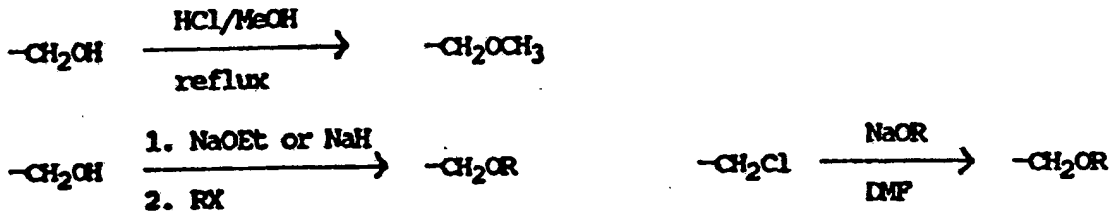
B. $-NH(CO-O-^tButyl) \xrightarrow{TFA} -NH_2$

Boc (protecting group)

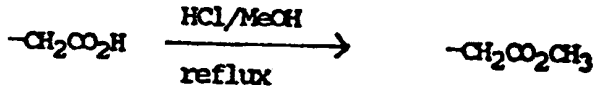
3. Tetrazole

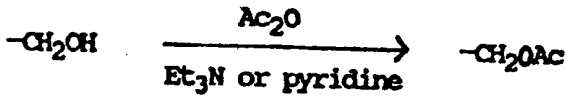


4. Ether

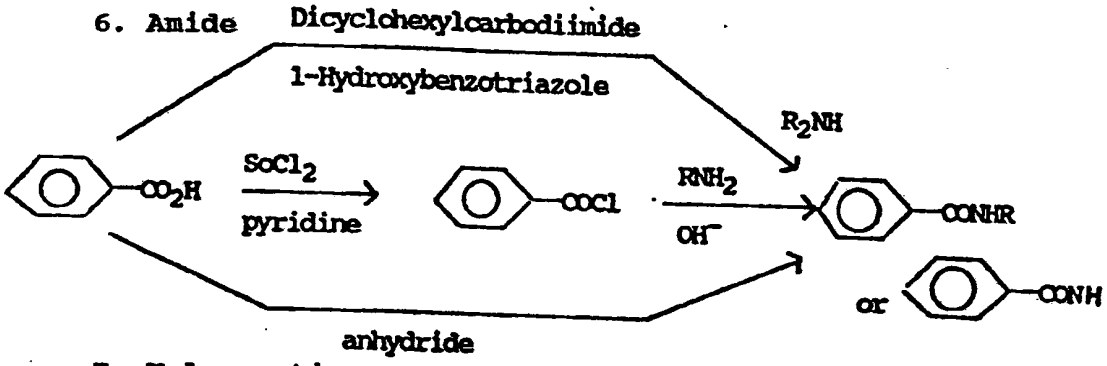


5. Ester

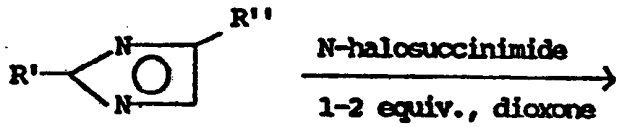


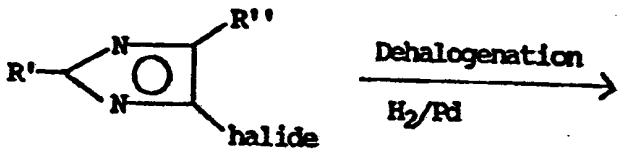


6. Amide

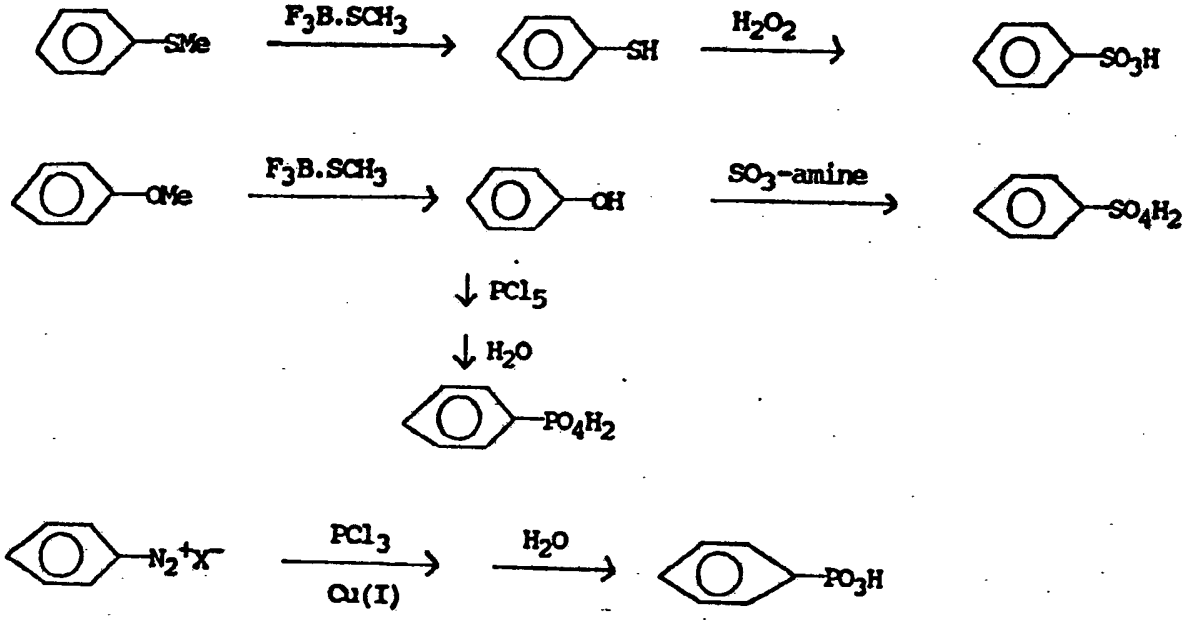


7. Halogenation

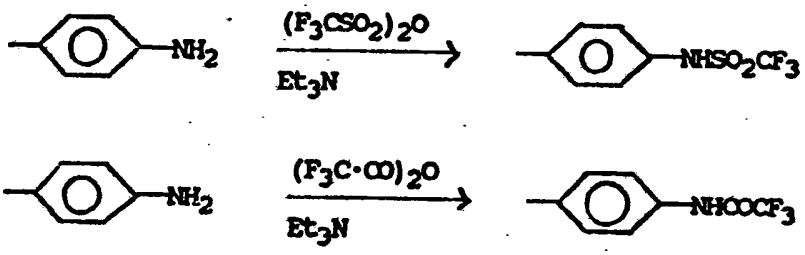




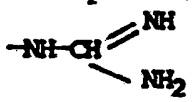
8. Sulfonation/Phosphonation



9. Trifluoromethylsulphonamido and Trifluoromethylacetamido

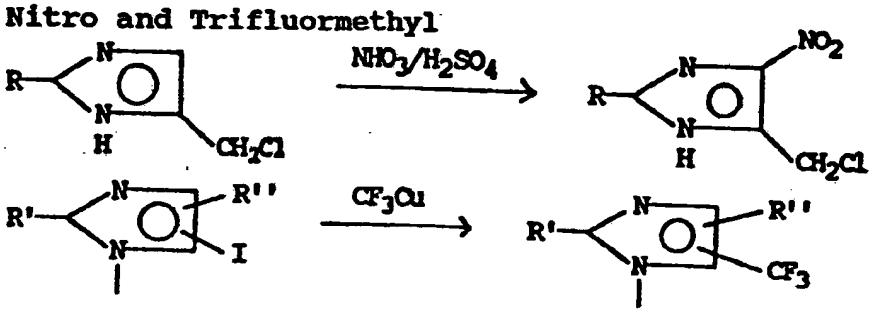


10. Protecting Groups for -NH, $-NH_2$, -OH, $-CO_2H$, $-CONH_2$, or

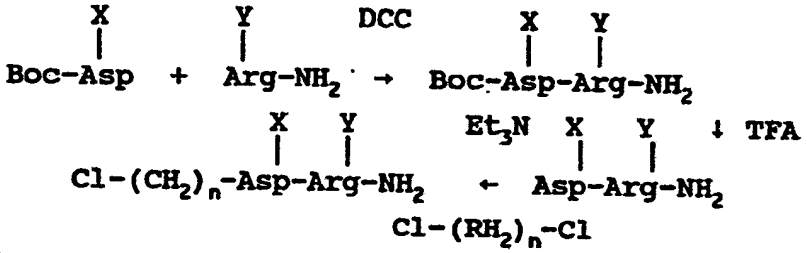


Acid-labile and base-labile protecting groups and protecting groups removable by hydrogenation are well known in the art. Methods for introducing such protecting groups and for their removal are familiar to one skilled in the art as set forth in J. Stewart and J. Young, Solid Phase Peptide Synthesis, 1984, Pierc Chem. Co.

11. Nitro and Trifluormethyl



12. $-(CH_2)_n-Asp-Arg-NH_2$



where X=benzyl, t-butyl Y=tosyl, nitro

In the above reaction, peptide should be added to large excess of cross-linker to avoid dimerization.

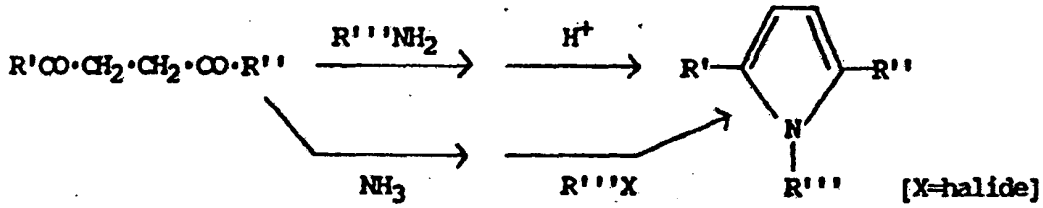
Non-imidazole Compounds

As previously indicated, it is possible to employ non-imidazole compounds as new antagonists. Such compounds are prepared by procedures which are analogous to those described above which provide substituents on the ring which can be converted to other groups. Details of methods and strategies for these syntheses have been extensively reviewed in European Patent 0,323,841 for compounds similar to the compounds of the present invention. Consequently, the following is a summary of the more important strategies available to obtain the required compounds, and is not intended to cover the entire field. As with substituted imidazoles, the elaboration of heterocyclic rings derivatized at the ring produces often a mixture of products which can be separated by conventional

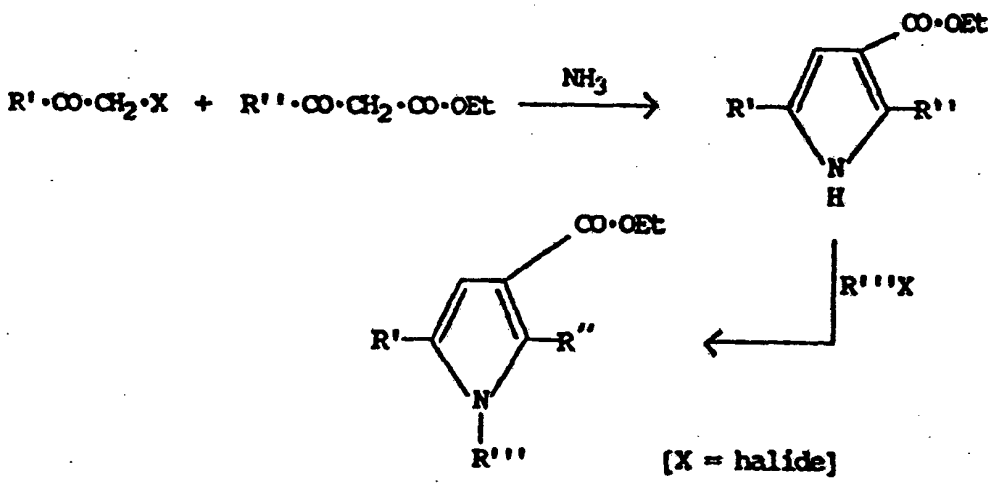
chromatography methods, and the isomers individually identified by Nuclear Overhauser Effect spectroscopy, and in some cases by bioassay or binding assay, i.e., the ability to displace angiotensin II from its receptor site.

Substituted Pyrole

1. Paal-Knorr reaction: condensation of 1,4 dicarbonyl compounds with ammonia or primary amine, as follows:

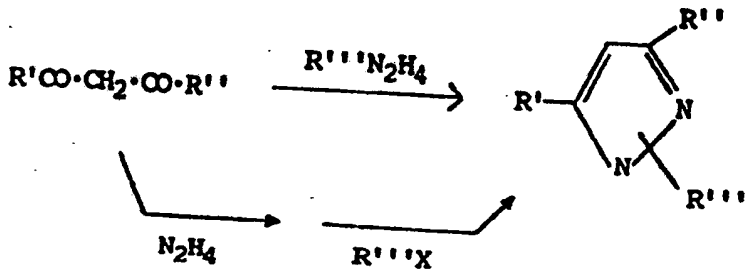


2. Hantzsch synthesis: condensation of α-haloketones (or α-hydroxyaldehyde or nitroalkenes) with ß-ketoesters in the presence of ammonia:



Substituted Pyrazole:

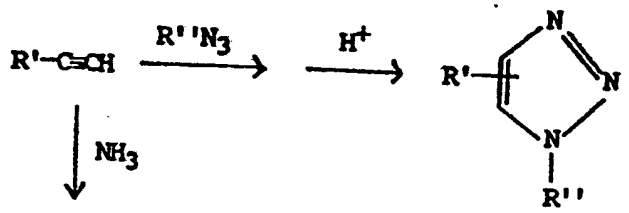
Condensation of 1,3 dicarbonyl compounds with hydrazine or its derivatives:



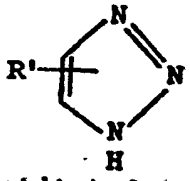
[X=halide]

Substituted 1,2,3-triazole

Thermal cycloaddition of azides to alkynes:



[1,4 substitution is favored but 1,5 isomer also formed]



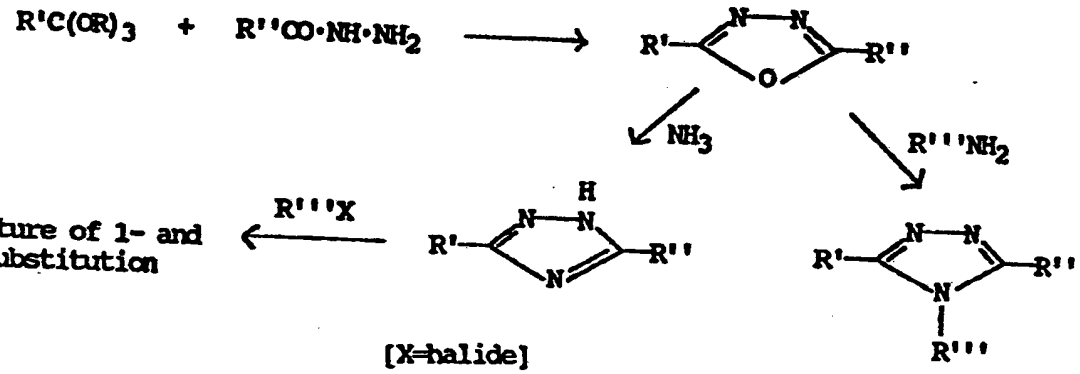
R''X

mixture of 1-, 2-, and 3-substitution

[X=halide]

Substituted 1,2,4-triazole

Reaction of orthoester and acylhydrazine to give 1,2,4-oxadiazole followed by reaction with ammonia or primary amine:



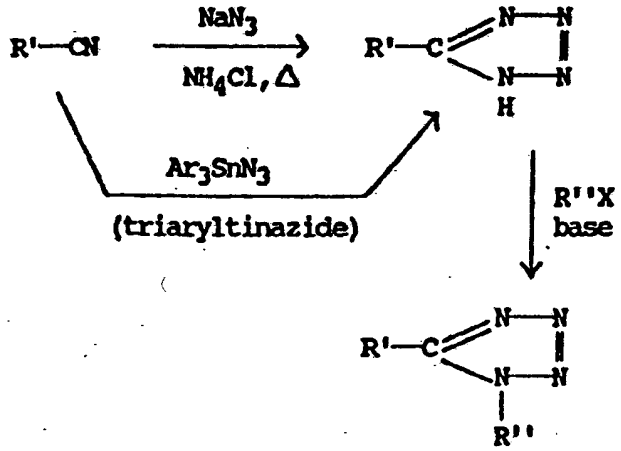
mixture of 1- and 2-substitution

[X=halide]

4-substituted

Substituted Tetrazole

General methods elaborated previously may be applied as set forth concerning protection of tetrazole:



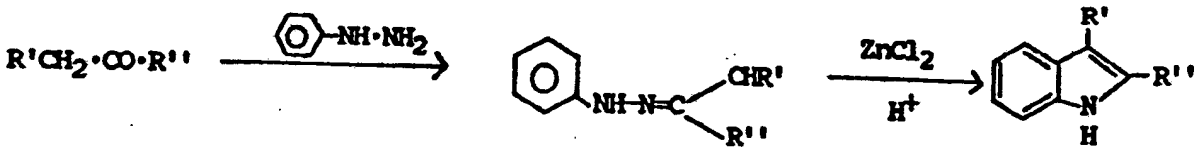
Protection of tetrazole may be accomplished using a trityl group if required or it may be otherwise substituted accordingly. [X=halide].

Substituted Pyridines, Diazines and Triazines are produced by analogous methods.

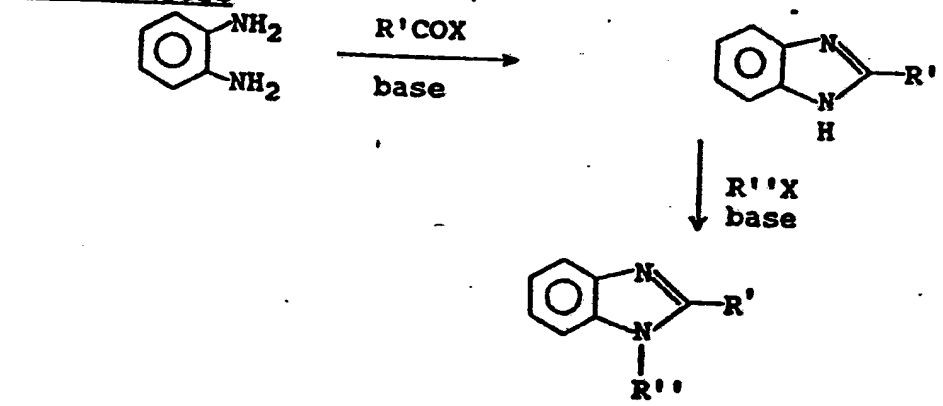
Polynuclear Heterocyclic Compounds

Substituted Indoles, Benzimidazole, Benzopyrazole and Benzotriazole

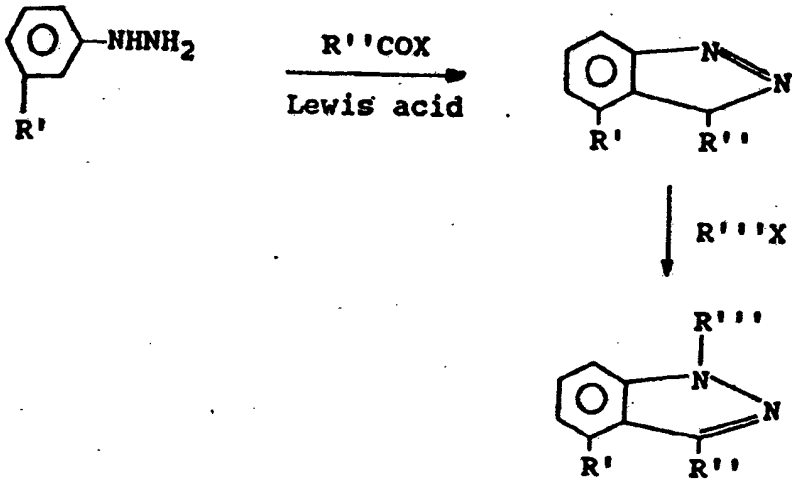
Fischer indole synthesis: arylhydrazones of aldehydes or ketones as treated with a catalyst such as $ZnCl_2$



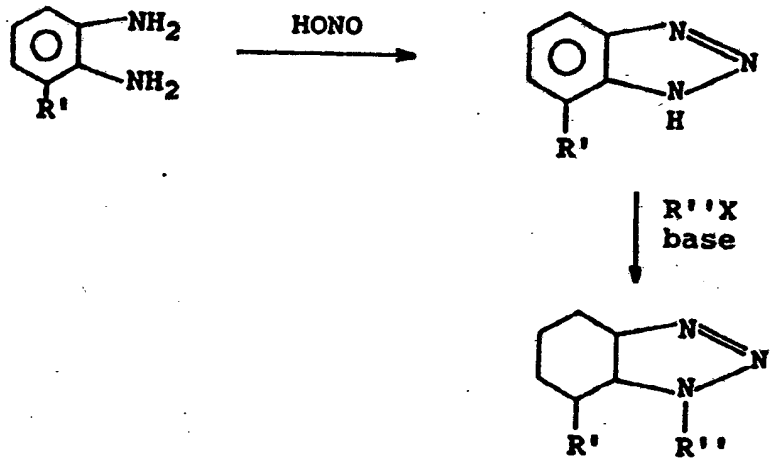
Benzimidazole



Benzopyrazole



Benzotriazole



Substituted Benopyridines, Benzodiazines and Benzotriazines are produced by analogous methods.

Compound Synthesis

The choice of starting materials and strategy of synthesis of a given compound is dictated by a number of factors including feasibility and cost. Th choice of methods and protecting groups and precurs r groups for a given synthesis is largely determined by other compromising structural and chemistry factors, which can be satisfactorily ascertained by one skilled in the art.

WHAT IS CLAIMED IS:

1. A method for creating a three-dimensional spatial model for a biologically active ligand having one or more active sites based on a charge-transfer interaction and further having a known structural formula wherein the three-dimensional spatial assignments for each of the atoms of the ligand in the model are assigned from the steps comprising:

a) determining the presence of charge-transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment;

b) determining the chemical groups involved in said charge-transfer interaction(s); and

c) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to the active site(s) from nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000.

2. A method according to Claim 1 which further comprises refining the three-dimensional model so generated by use of theoretical considerations.

3. A method according to Claim 1 which further comprises creating new three-dimensional models for said ligand by use of theoretical consideration.

4. A method according to Claim 1 wherein said biologically active ligand is selected from the group consisting of Angiotensin II, oxytocin, and vasopressin.

5. A method according to Claim 1 wherein said charge-transfer interaction is a tyrosinate charge-transfer interactions.

6. A method according to Claim 5 wherein said ligand has one active site based on a tyrosinate charge-transfer interaction.

7. A method according to Claim 6 wherein said ligand is Angiotensin II.

8. A method according to Claim 7 wherein the nuclear Overhauser effect technique is ROESY.

9. A method according to Claim 1 wherein fluorescence compatible environment is selected from the group consisting of micelles, lipid bilayers, and solvents having a dielectric constant of about 40 or less.

10. A method according to Claim 1 wherein said nuclear Overhauser effect technique is NOESY.

11. A method according to Claim 1 wherein said nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect is conducted in a receptor-simulating environment.

12. A method according to Claim 1 wherein said ligand is complementary to a membrane bound biologically active receptor.

13. A method according to Claim 12 wherein said nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect is conducted in a solvent having a dielectric constant of about 50 or less.

14. A method according to Claim 13 wherein said solvent is dimethylsulfoxide.

15. A method of modelling antagonists to a biologically active receptor based on the model generated for a biologically active ligand complementary to said receptor wherein said ligand has one or more active sites based on a charge-transfer interaction and further has a known structural formula which method comprises the steps of:

a) creating a three-dimensional spatial model for said ligand by

i) determining the presence of charge-transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment;

ii) determining the chemical groups involved in said charge-transfer interaction(s); and

iii) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to th active site(s) from nucl ar

magnetic resonance spectroscopy employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000; and

b) identifying a compound having a three-dimensional structure sufficiently similar to said ligand so as to be complementary to said receptor and wherein at least one of the charge-transfer interactions in said compound has been compromised.

16. A method according to Claim 15 wherein said biologically active ligand is the naturally occurring biologically active ligand complementary for said receptor.

17. A method according to Claim 15 wherein said biologically active ligand has only one charge-transfer interaction.

18. A method according to Claim 16 wherein said naturally occurring biologically active ligand is selected from the group consisting of Angiotensin II, oxytocin and vasopressin.

19. A method according to Claim 18 wherein said nuclear overhauser effect is ROESY.

20. A method according to Claim 15 wherein said biologically active receptor is a membrane bound rec ptor.

21. A method of modelling agonists to a biologically active receptor based on the model generated for a biologically active ligand complementary to said receptor wherein said ligand has one or more active sites based on a charge-transfer interaction and further has a known structural formula which comprises the steps of:

a) creating a three-dimensional spatial model for said ligand by

i) determining the presence of charge transfer interaction(s) in said ligand from fluorescence analysis of said ligand in a fluorescence compatible environment; and

ii) determining the chemical groups involved in said charge-transfer interaction(s); and

iii) resolving remaining aspects of the ligand's three-dimensional conformation by obtaining conformational information relative to the active site(s) from nuclear magnetic resonance spectroscopy employing the nuclear Overhauser effect providing that when the nuclear Overhauser effect technique employed in this step is NOESY, then the molecular weight of said ligand is either less than about 500 or greater than about 2000; and

b) identifying a compound having a three-dimensional structure sufficiently similar to said ligand so as to be complementary to said receptor and

wh rein the charge-transfer interaction(s) in said compound has (have) not been compromised.

22. A method according to Claim 21 wherein said biologically active ligand is the naturally occurring biologically active ligand complementary to said receptor.

23. A method according to Claim 22 wherein said naturally occurring biologically active ligand has only one charge-transfer interaction.

24. A method according to Claim 23 wherein said naturally occurring biologically active ligand is selected from the group consisting of Angiotensin II, oxytocin and vasopressin.

25. A method according to Claim 24 wherein said nuclear Overhauser effect is ROESY.

26. A method according to Claim 21 wherein said biologically active receptor is a membrane bound receptor.

27. A three-dimensional model for Angiotensin II as depicted in FIGURE 6.

28. A method for modelling an antagonist to Angiotensin II which comprises creating a compound having a three-dimensional structure sufficiently similar to the assignment for Angiotensin II defined in Claim 27 so as to be complementary to the biologically active receptor for Angtiotensin II and wherein the

charge-transfer interaction in said compound has been compromised.

29. A method for modelling an agonist to Angiotensin II which comprises creating compounds having a three-dimensional structure sufficiently similar to the assignment for Angiotensin II defined in Claim 27 so as to be complementary to the biologically active receptor for Angiotensin II and wherein the charge-transfer interaction in said compound has not been compromised.

30. A three-dimensional model for receptor bound Angiotensin II as depicted in FIGURE 8B.

31. A method for determining the presence of charge-transfer interaction(s) in the tertiary structure of a biologically active ligand complementary to a biologically active receptor which comprises conducting fluorescence analysis of said ligand in a fluorescence compatible environment.

32. A method according to Claim 31 wherein said fluorescence compatible environment comprises micelles, lipid bilayers, and solvents having a dielectric constant of less than about 40.

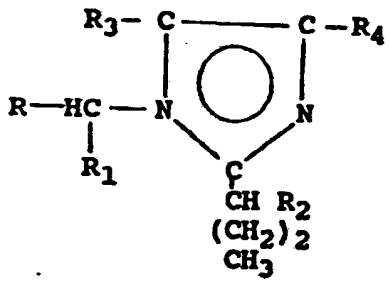
33. A method according to Claim 31 wherein said biologically active ligand has only one charge-transfer interaction.

34. A method according to Claim 33 wherein said biologically active ligand is the naturally occurring biologically active ligand.

35. A method according to Claim 34 wherein said naturally occurring biologically active ligand is selected from the group consisting of Angiotensin II, oxytocin and vasopressin.

36. A method according to Claim 31 wherein said biologically active receptor is a membrane bound receptor.

37. A compound of the formula



wherein R is selected from the group consisting of phenyl para substituted with a substituent selected from the group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido, and $-NHC(O)R_5$ wherein R_5 is phenyl ortho substituted with a substituent selected from the group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido; wherein R_1, R_2, R_3 and/or R4 provide one or more of the following charges:

i) a cationic charge at a direction left of the center of the imidazole ring at a distance of about 7 ± 1.5 Angstroms;

ii) an anionic charge at a direction right of the center of the imidazole ring at a distanc of about 2.5 ± 0.5 Angstroms;

iii) an anionic charge at a direction left of the center of the imidazole ring at a distance of about 10 ± 2 Angstroms; and

iv) a cationic charge at a direction left of the center of the imidazole ring at a distance of about 12 ± 2.5 Angstroms; providing that when any of R_1, R_2, R_3, and R_4 are not providing such an ionic charge then R_1 is hydrogen, R_2 is hydrogen, R_3 is either hydroxymethyl, $-CH_2-O-CH_3$, $-CH_2C(O)OCH_3$ or $-C(O)OCH_3$ and R_4 is fluorine or chlorine, further providing that said compound contains no more than one charge for each of i), ii), iii) and iv), and still further providing that the placement of such charges left and/or right of the imidazole ring is as defined in Formula I and II of this application.

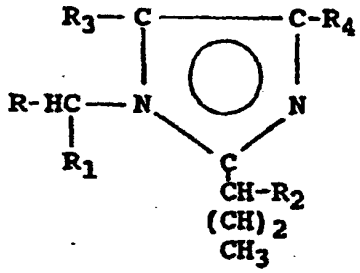
38. A pharmaceutical composition which comprises a pharmaceutically acceptable carrier and an effective amount to control hypertension in a mammal in need of such treatment of a compound defined in Claim 37.

39. A pharmaceutical composition which comprises a pharmaceutically acceptable carrier and an effective amount to control congestive heart failure in a mammal in need of such treatment of a compound defined in Claim 37.

40. A method of controlling hypertension in a mammal in need of such treatment by administering either orally or parenterally a pharmaceutical composition as defined in Claim 38.

41. A method of controlling congestive heart failure in a mammal in need of such treatment by administering either orally or parenterally a pharmaceutical composition as defined in Claim 39.

42. A compound of the formula



wherein R is selected from the group consisting of a) phenyl para substituted with a substituent selected from the group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido, and b) -NHC(O)R_5 wherein R_5 is phenyl ortho substituted with a substituent selected from an acidic group consisting of carboxyl or a pharmaceutically acceptable salt thereof, sulfate, and trifluoromethylsulfonamido, wherein either R_1 or R_3 but not both provides a cationic charge left of the center of the imidazole ring at a distance of about 7 ± 1.5 Angstroms providing that when R_3 provides such a charge then R1 is hydrogen and further providing that when R_1 provides such a charge then R_3 is hydroxymethyl,

$-CH_2OCH_3$, $-CH_2CO_2CH_3$ or $-CO_2CH_3$ further wherein either R_2 or R_4 but not both provides an anionic charge right of the center of the imidazole ring at a distance of about 2.5 ± 0.5 Angstroms providing that when R_2 provides such a charge, then R4 is either fluorine or chlorine and further providing that when R_4 provides such a charge, then R_2 is hydrogen and still further providing that the placement of charges left and/or right of the imidazole ring is as defined in Formula I and II of this application.

43. A compound as defined in Claim 40 wherein R_1 is hydrogen and R_3 is selected from the group consisting of $-(CH_2)_4-NH_2$, $-CHOH-(CH_2)_3-NH_2$, and $-(CH_2)_4-Asp-Arg-NH_2$.

44. A compound as defined in Claim 40 wherein R_3 is hydroxymethyl, $-CH_2OCH_3$, $-CH_2CO_2CH_3$ or $-CO_2CH_3$ and R_1 is selected from the group consisting of $-(CH_2)_3-NH_2$ and $-(CH_2)_3-Asp-Arg-NH_2$.

45. A compound as defined in Claim 40 wherein R_2 is hydrogen and R_4 is $-(CH_2))_3-C(O)OH$.

46. A compound as defined in Claim 40 wherein R_4 is either hydrogen or chlorine and R_2 is $-(CH_2)_3-C(O)OH$.

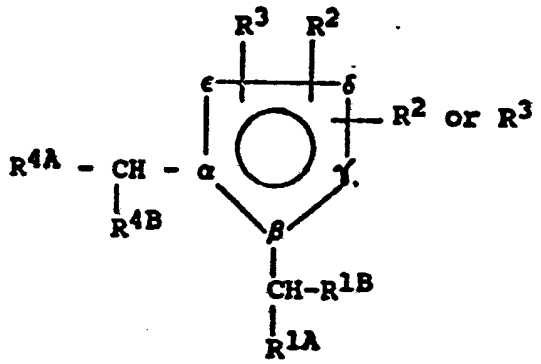
47. A pharmaceutical composition which comprises a pharmaceutically acceptable carrier and an effective amount to control hypertension in a mammal in need of such treatment of a compound as defined in Claim 42.

48. A pharmac utical composition which comprises a pharmaceutically acceptable carrier and an effective amount to control congestive heart failure in a mammal in need of such treatment of a compound as defined in Claim 42.

49. A method of controlling hypertension in a mammal in need of such treatment which comprises administering either orally or parenterally a pharmeceutical composition defined in Claim 47.

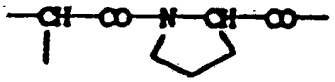
50. A method of controlling congestive heart failure in a mammal in need of such treatment which comprises administering either orally or parenterally a pharmeceutical composition defined in Claim 48.

51. A compound of the formula:



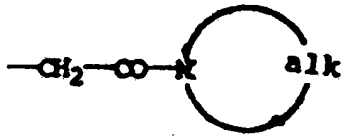
wherein α, β, γ, δ, and ε are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom and (b) attachment of R groups is to C or N;

R^{1A}, which mimics the structure in angiotensin of



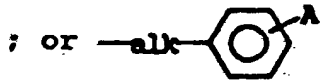
includes the following:

-alk; -O-alk; -alk-O-alk; $-CH_2-CO-NH_2$; $-CH_2-CO-NH-alk$; $-CH_2-CO-N(alk)_2$;

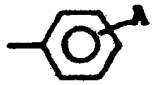
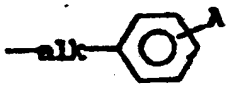


$-CH_2-CO-AA-NH_2$; or $-CH_2-CO-AA-Phe$, wherein AA is an amino acid;

R^{1B}, which provides a spacer arm terminating in a mimic of the C-terminal carboxylate group of angiotensin II, includes the following:

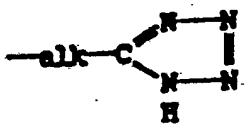
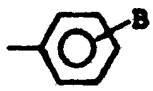
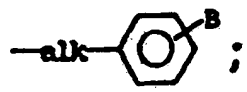
-H; -alk-A;  ; or  ;

R^2, which provides steric and/or electronic properties and/or a spacer arm terminating in an acid group, includes the following: -H, -halide; -alk; -O-alk; $-NO_2$; $-CF_3$; -CN; -alk-A; -A;

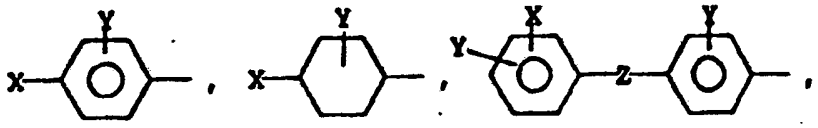
 ; or 

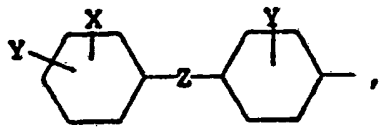
R^3, which provides steric and/or electronic properties and/or a mimetic of the tyrosine hydroxyl group of angiotensin II in its "charge relay" conformation, or a spacer arm terminating in a mimic of the N-terminus of N-terminal dipeptide of angiotensin-II, includes the following;

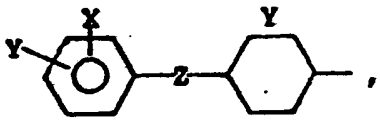
-H; -alk; -aryl; -alk-OH; -alk-halide; $-CH_2-O-alk$; $-CH_2-CN$; $-CH_2-CO_2H$; $-CH_2CO_2-alk$; -NH-CO-alk; -CO-NH-alk; -alk-B; -CH(OH)-alk-B; -alk-Asp-Arg-NH_2; -CH(OH)-alk-Asp-Arg-NH_2;

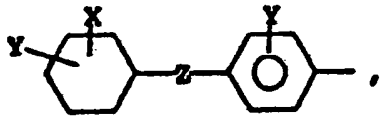
 ;  ; or  ;

R^{6A}, which provides a spacer arm, the relative rigidity of which is an aspect of the design, terminating in an acid group which mimics the tyrosine hydroxy groups of angiotensin II in its "receptor bound" conformation, or a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin II, includes the following:

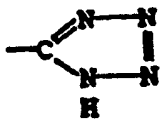






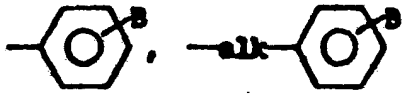


where Z is a bond, -NHCO-, -O-, $-OCH_2-$, or $-CH_2-$;
X is $-CO_2H$, -alk-CO_2H, $-PO_3H$, -alk-PO_3H, $-PO_4H_2$, -alk-PO_4H_2, -SH, -alk-SH, $-SO_3H$, -alk-SO_3H, $-SO_4H_2$, -alk-SO_4H_2, $F_3C-CO-NH-$, F_3C-SO_2-NH-,



or a pharmaceutically acceptable salt thereof; and
Y is -H, -halide, $-NO_2$, -O-alk, -alk, $-CF_3$, or -CN; and

R^{4B}, which provides a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin, includes the following:
-H, -alk-B, -alk-Asp-Arg-NH_2, -alk-O-alk-B,
-alk-O-alk-Asp-Arg-NH_2,



alk = an alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having 3-6 carbon atoms, an alkenyl group having 2-10 carbon atoms, or an alkynyl group having 2-10 carbon atoms;

halide = -F, -Cl, -Br, or -I;

A = an acid group or its pharmaceutical salt; and

B = a basic group or its pharmaceutical salt.

52. A compound of Claim 51 wherein when R^{1B} is H, then: (a) if the ring is imidazole either α or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2.

53. A compound of Claim 51 wherein when R^{4B} is H, then: (a) if the ring is imidazole either α or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2.

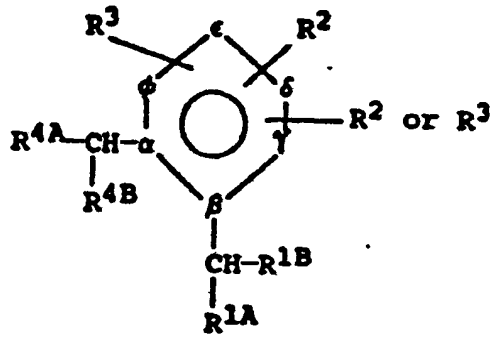
54. A compound of Claim 51 wherein the ring is imidazole, pyrole, pyrrazole, 1,2,3- and 1,2,4-triazole, tetrazole or thiazole, oxazole, thiadiazole, or oxadiazole.

55. A compound of Claim 54 wherein the ring is an imidazole.

56. A compound of Claim 51 wherein at least one of the substitutents R^2 or R^3, which are bonded to the ring atoms γ, δ, or ϵ is hydrogen.

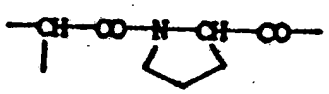
57. A compound of Claim 51 wherein R^{1B} or R^{4B} is hydrogen.

58. A compound of the formula:



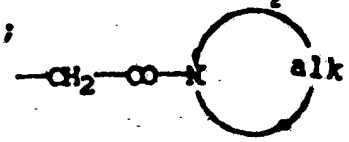
wherein α, β, γ, δ, ϵ, and ϕ are C, N, O or S with the provisos that (a) the ring contains at least one C atom and one N atom and (b) attachment of R groups is to C or N;

R^{1A}, which mimics the structure in angiotensin f



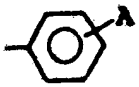
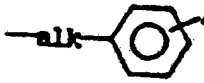
includes the following:

-alk; -O-alk; -alk-O-alk; $-CH_2-CO-NH_2$; $-CH_2-CO-NH-alk$; $-CH_2-CO-N(alk)_2$;

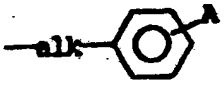


$-CH_2-CO-AA-NH_2$; or $-CH_2-CO-AA-Phe$,
wherein AA is an amino acid;

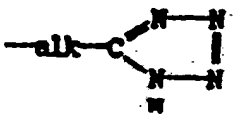
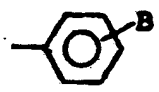
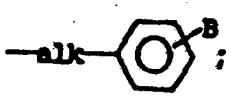
R^{1B}, which provides a spacer arm terminating in a mimic of the C-terminal carboxylate group of angiotensin II, includes the following:

-H; -alk-A;  ; or  ;

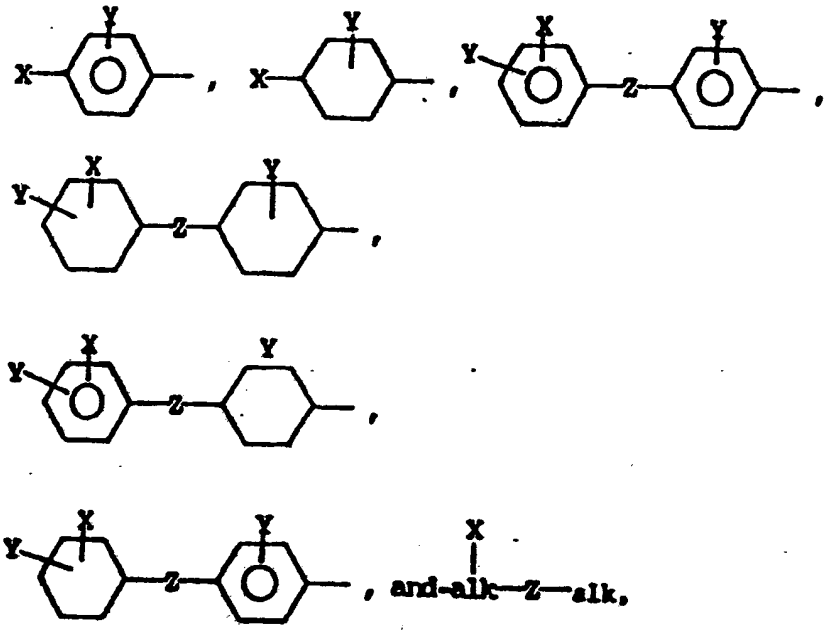
R^2, which provides steric and/or electronic properties and/or a spacer arm terminating in an acid group, includes the following: -H, -halide; -alk; -O-alk; $-NO_2$; $-CF_3$; -CN; -alk-A; -A;

 ; or 

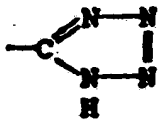
R^3, which provides steric and/or electronic properties and/or a mimetic of the tyrosine hydroxyl group of angiotensin II in its "charge relay" conformation, or a spacer arm terminating in a mimic of the N-terminus of N-terminal dipeptide of angiotensin-II, includes the following;
-H; -alk; -aryl; -alk-OH; -alk-halide; $-CH_2-O-alk$; $-CH_2-CN$; $-CH_2-CO_2H$; $-CH_2CO_2-alk$; -NH-CO-alk; -CO-NH-alk; -alk-B; -CH(OH)-alk-B; -alk-Asp-Arg-NH_2; -CH(OH)-alk-Asp-Arg-NH_2;

 ;  ; or  ;

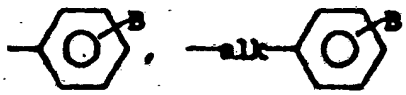
R^{4A}, which provides a spacer arm, the relative rigidity of which is an aspect of the design, terminating in an acid group which mimics the tyrosine hydroxy groups of angiotensin II in its "receptor bound" conformation, or a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin II, includes the following:



where Z is a bond, -NHCO-, -O-, $-OCH_2-$, or $-CH_2-$;
X is $-CO_2H$, -alk-CO_2H, $-PO_3H$, -alk-PO_3H, $-PO_4H_2$, -alk-PO_4H_2, -SH, -alk-SH, $-SO_3H$, -alk-SO_3H, $-SO_4H_2$, -alk-SO_4H_2, $F_3C-CO-NH-$, F_3C-SO_2-NH-,



or a pharmaceutically acceptable salt thereof; and
Y is -H, -halide, $-NO_2$, -O-alk, -alk, $-CF_3$, or -CN; and
R^{4B}, which provides a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin, includes the following:
-H, -alk-B, -alk-Asp-Arg-NH_2, -alk-O-alk-B, -alk-O-alk-Asp-Arg-NH_2,



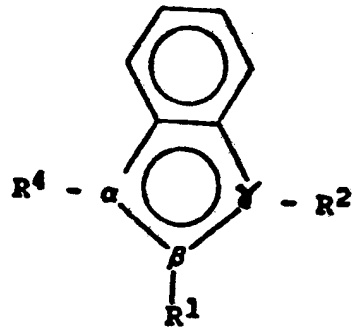
alk = an alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having 3-6 carbon atoms, an alkenyl group having 2-10 carbon atoms, or an alkynyl group having 2-10 carbon atoms;
halide = -F, -Cl, -Br, or -I;
A = an acid group or its pharmaceutical salt; and
B = a basic group or its pharmaceutical salt.

59. A compound of Claim 58 wherein when R^{1B} is H, then: (a) if the ring is imidazole either α or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 compris s a group containing B or -Asp-Arg-NH_2.

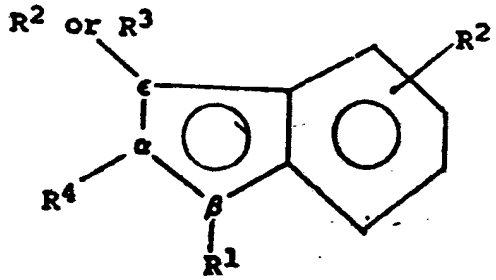
60. A compound of Claim 58 wherein when R^{4B} is H, then: (a) if the ring is imidazole either α or γ is other than N, (b) if the ring is other than imidazole either α is C or β is N, (c) R^{1A} comprises a group containing an amide, (d) R^2 comprises a group containing A, or (e) R^3 comprises a group containing B or -Asp-Arg-NH_2;

61. A compound of Claim 56 wherein at least one of the substitutents R^2 or R^3, which are bonded to the ring atoms γ, δ, or ϵ is hydrogen.

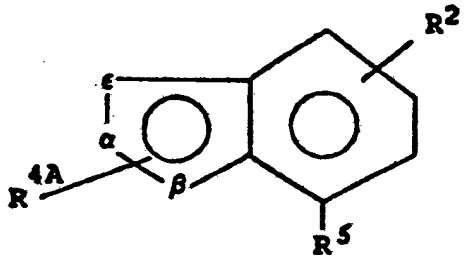
62. A compound of the formula:



(I)



(II)

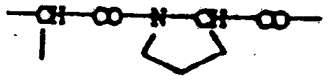


(III)

wherein α, β, and γ are C or N, with the provisos that only one N atom is substituted;

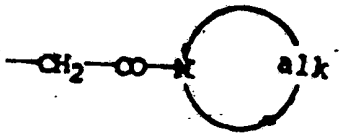
R^1 is $-CH(R^{1A})(R^{1B})$ wherein

R^{1A}, which mimics the structure in angiotensin of



includes the following:

-alk; -O-alk; -alk-O-alk; $-CH_2-CO-NH_2$; $-CH_2-CO-NH-alk$; $-CH_2-CO-N(alk)_2$;



$-CH_2-CO-AA-NH_2$; or $-CH_2-CO-AA-Phe$,

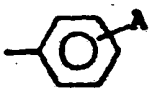
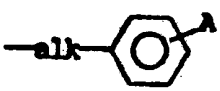
wherein AA is an amino acid, azetidine-carboxylic acid, pipecolic acid, nipecotic acid, glycine, alanine, sarcosine, or N-methyl-alanine;

R^{1B}, which provides a spacer arm terminating in a mimic of the C-terminal carboxylate group of angiotensin II, includes the following:

-H; -alk-A;  ; or  ;

with the proviso that when R^{1B} is H then (a) R^{1A} comprises a group containing an amide, or (b) R^1 is on an N, or (c) R^4 is on a C;

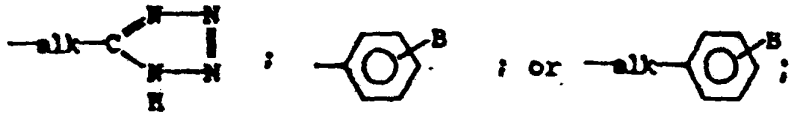
R^2, which provides steric and/or electronic properties and/or a spacer arm terminating in an acid group, includes the following: -H, -halide; -alk; -O-alk; $-NO_2$; $-CF_3$; -CN; -alk-A; -A;

 ; or 

R^3, which provides st ric and/or electronic properties and/or a mimetic f the tyrosine hydroxyl

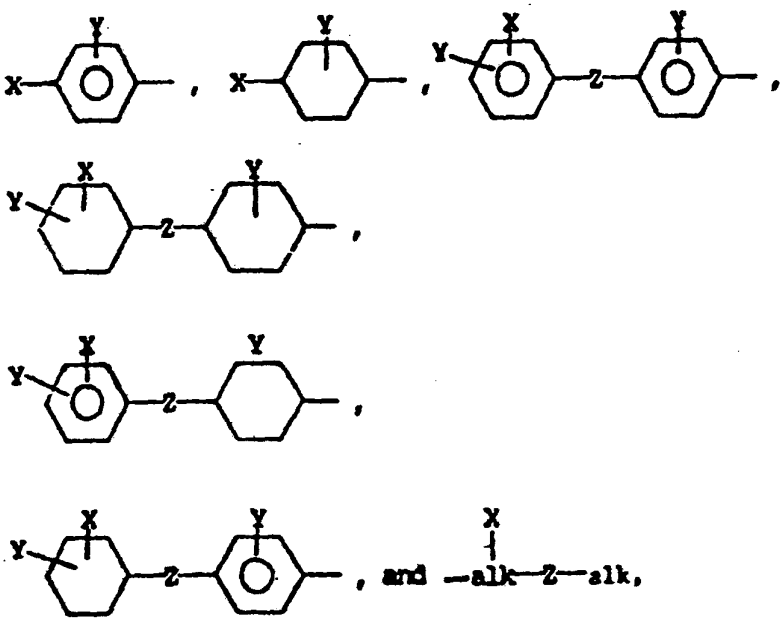
group of angiotensin II in its "charge relay" conformation, or a spacer arm terminating in a mimic of the N-terminus of N-terminal dipeptide of angiotensin-II, includes the following;

-H; -alk; -aryl; -alk-OH; -alk-halide; $-CH_2-O-alk$; $-CH_2-CN$; $-CH_2-CO_2H$; $-CH_2CO_2-alk$; -NH-CO-alk; -CO-NH-alk; -alk-B; -CH(OH)-alk-B; -alk-Asp-Arg-NH_2; -CH(OH)-alk-Asp-Arg-NH_2;

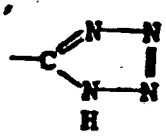


R^4 is $-CH(R^{4A})(R^{4B})$ wherein

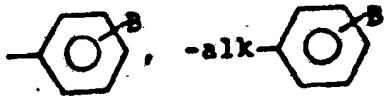
R^{4A}, which provides a spacer arm, the relative rigidity of which is an aspect of the design, terminating in an acid group which mimics the tyrosine hydroxy groups of angiotensin II in its "receptor bound" conformation, or a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin II, includes the following:



where Z is a bond, -NHCO-, -O-, $-OCH_2-$, or $-CH_2-$;
X is $-CO_2H$, -alk-CO_2H, $-PO_3H$, -alk-PO_3H, $-PO_4H_2$, -alk-PO_4H_2, -SH, -alk-SH, $-SO_3H$, -alk-SO_3H, $-SO_4H_2$, -alk-SO_4H_2, $F_3C-CO-NH-$, F_3C-SO_2-NH-,



or a pharmaceutically acceptable salt thereof; and
Y is -H, -halide, $-NO_2$, -O-alk, -alk, $-CF_3$, or -CN; and
R^{4B}, which provides a spacer arm terminating in a mimic of the N-terminus or N-terminal dipeptide of angiotensin, includes the following:
-H, -alk-B, -alk-Asp-Arg-NH_2, -alk-O-alk-B, -alk-O-alk-Asp-Arg-NH_2,



R^5 is the same as R^1 defined above;
alk = an alkyl group having from 1 to 10 carbon atoms, a cycloalkyl group having 3-6 carbon atoms, an alkenyl group having 2-10 carbon atoms, or an alkynyl group having 2-10 carbon atoms;
halide = -F, -Cl, -Br, or -I;
A = an acid group or its pharmaceutical salt; and
B = a basic group or its pharmaceutical salt.

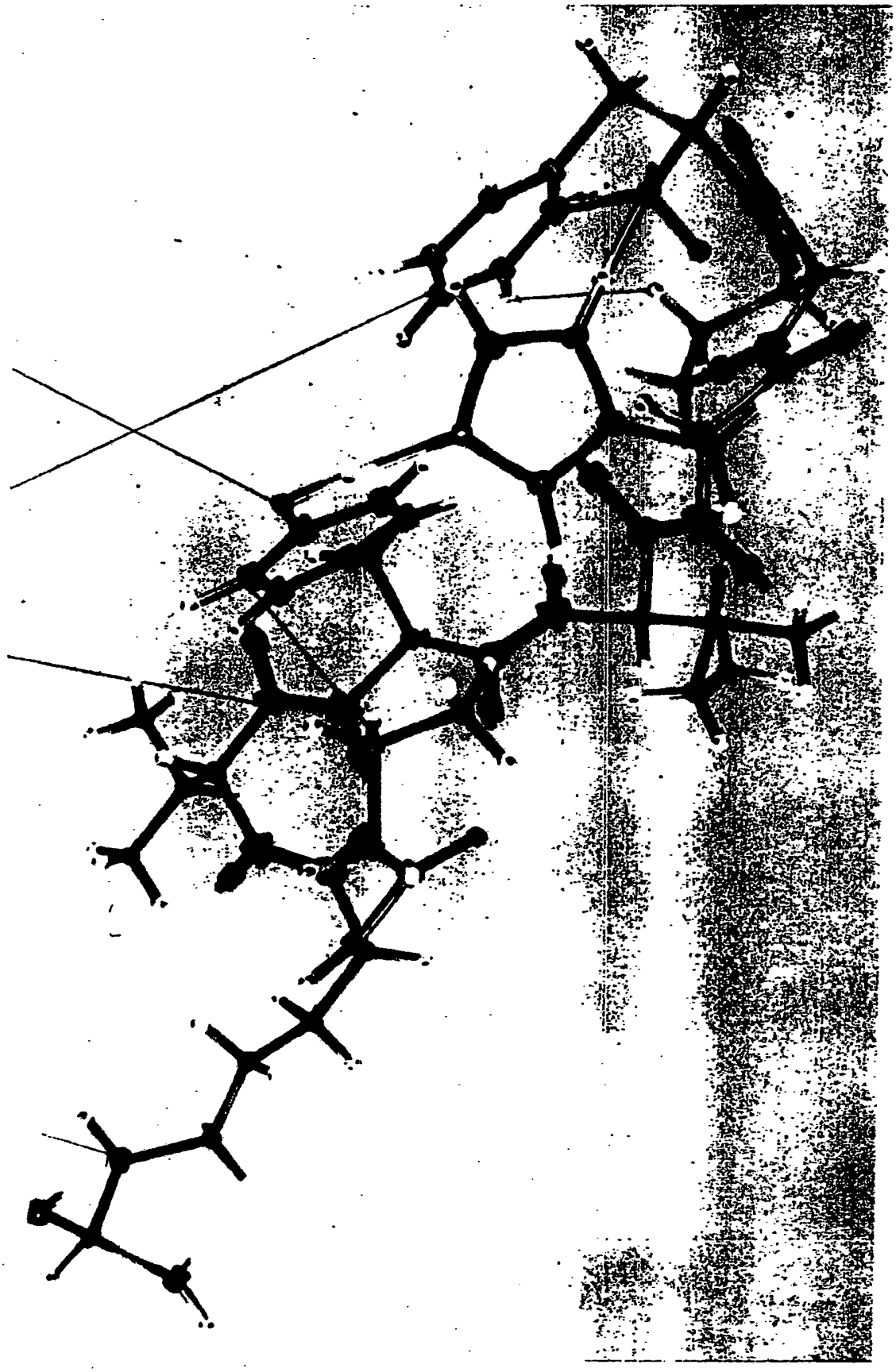


FIG._1

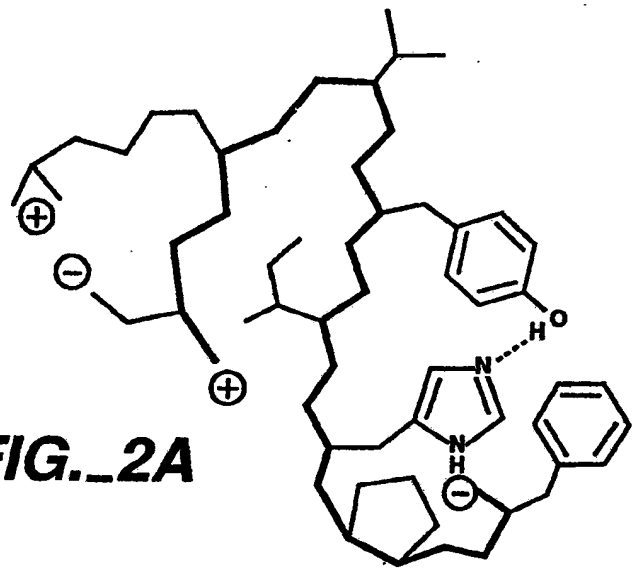


FIG._2A

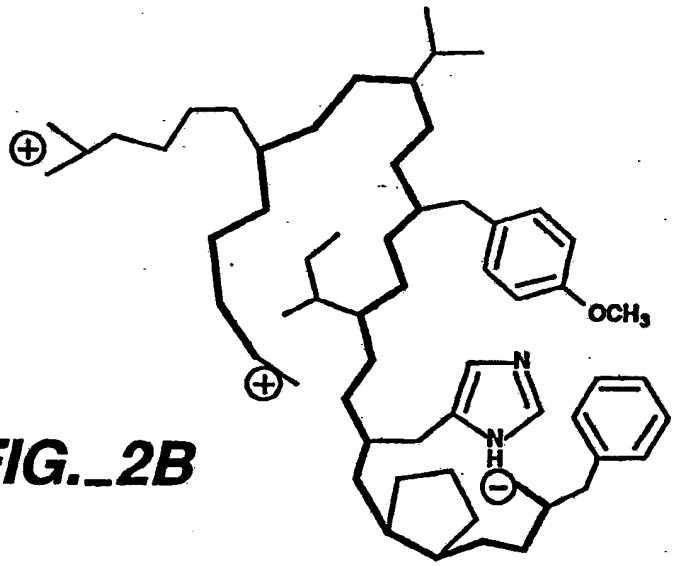


FIG._2B

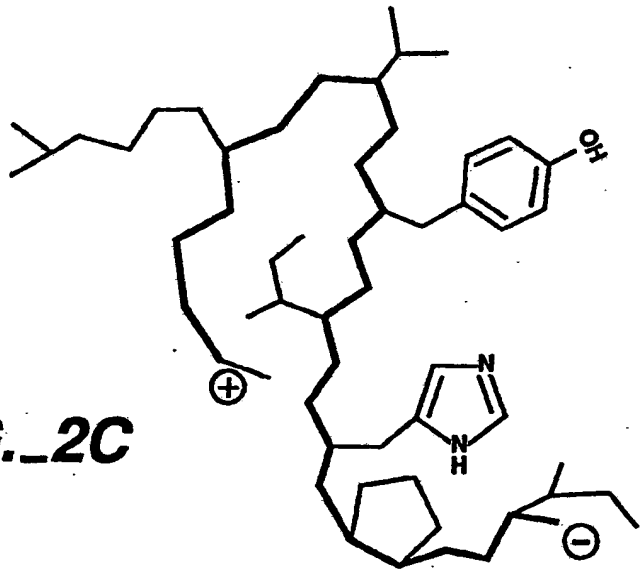


FIG._2C

3/10

BI

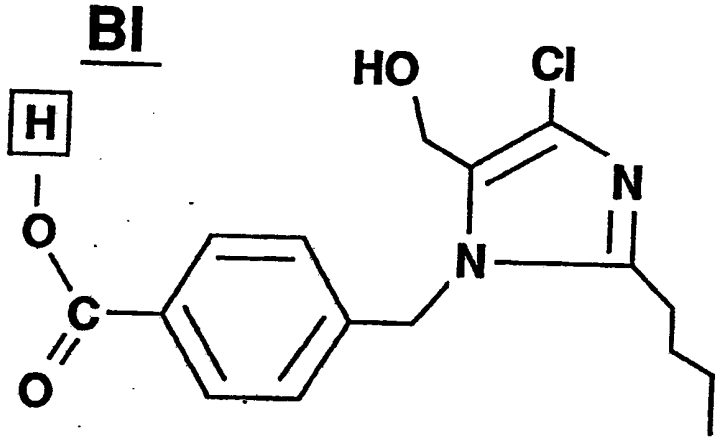


FIG._3A

BABI

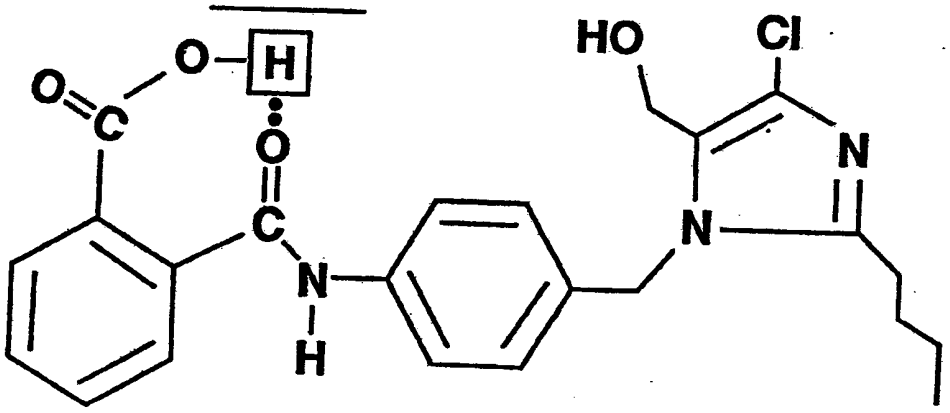


FIG._3B

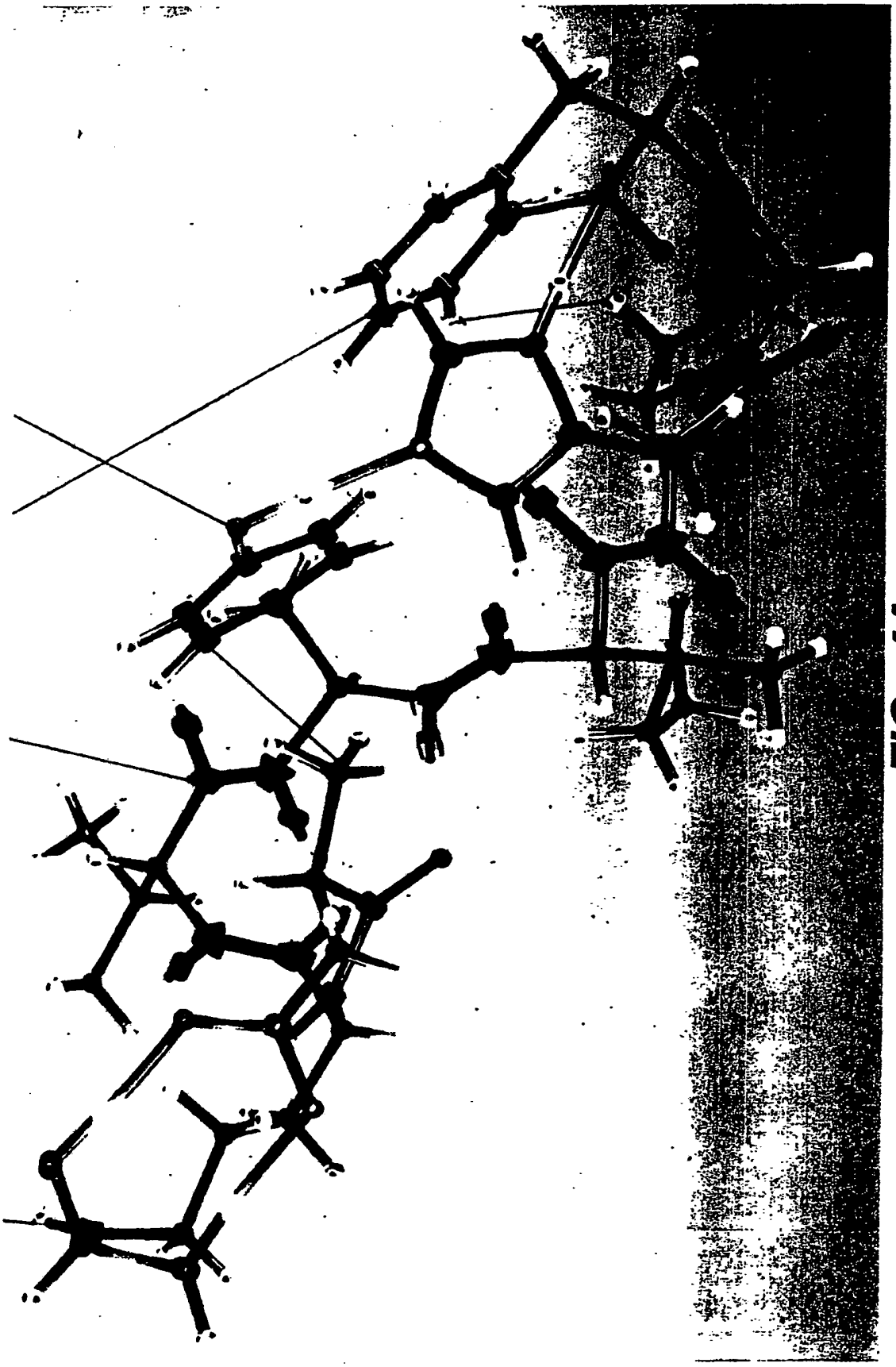


FIG._4A

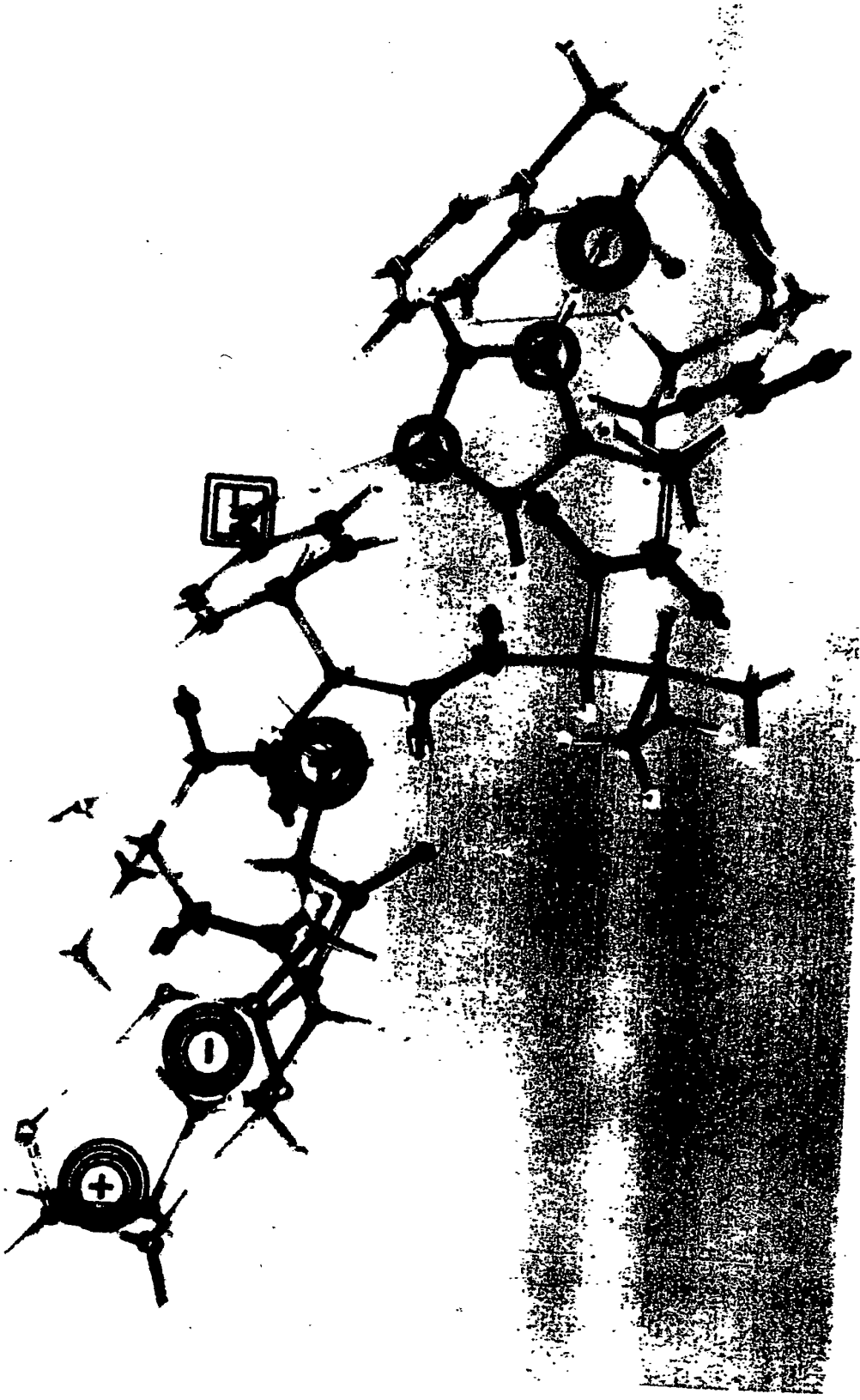


FIG._4B

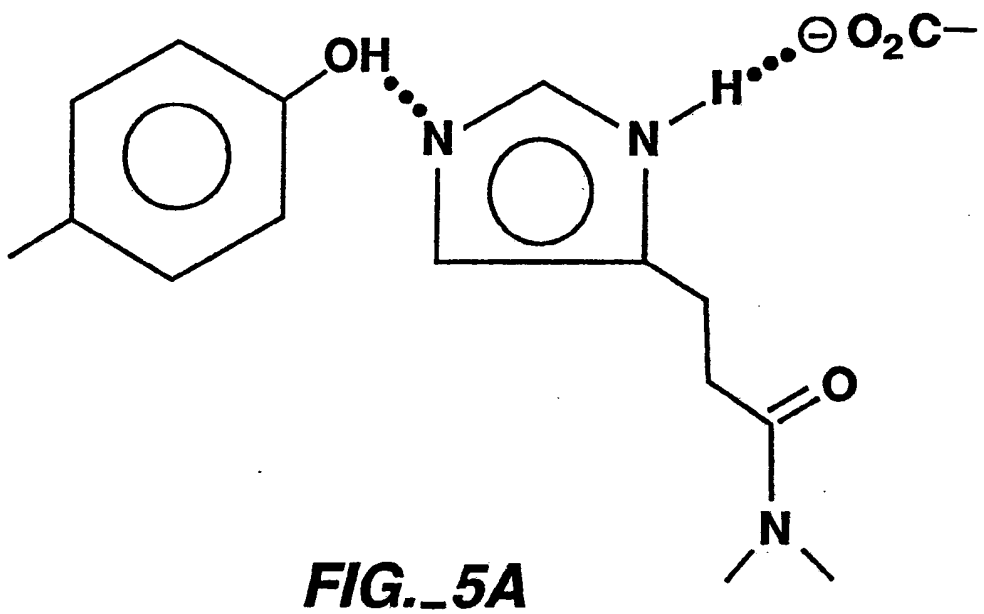


FIG._5A

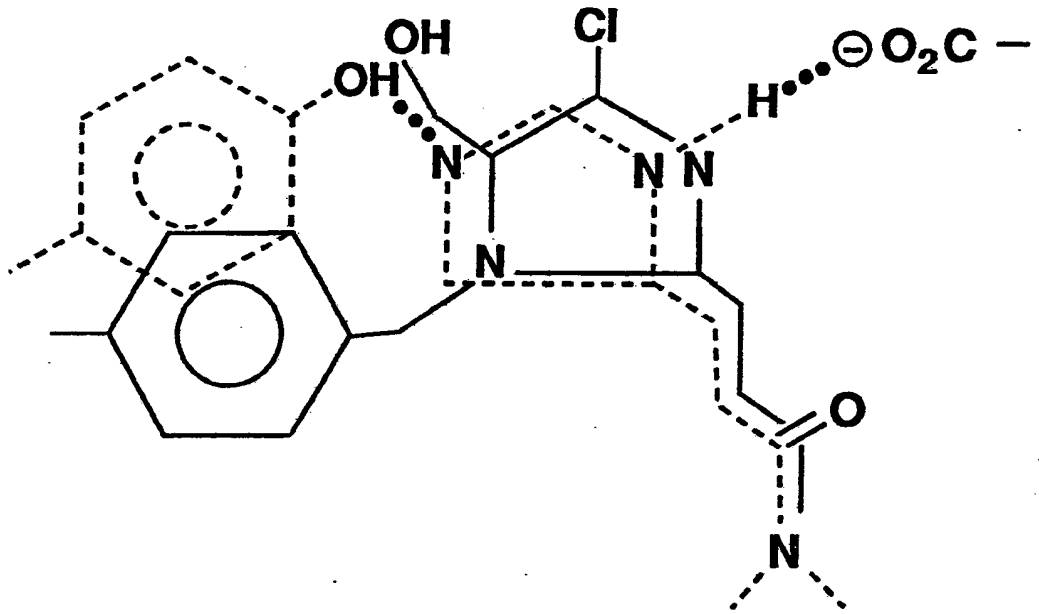


FIG._5B

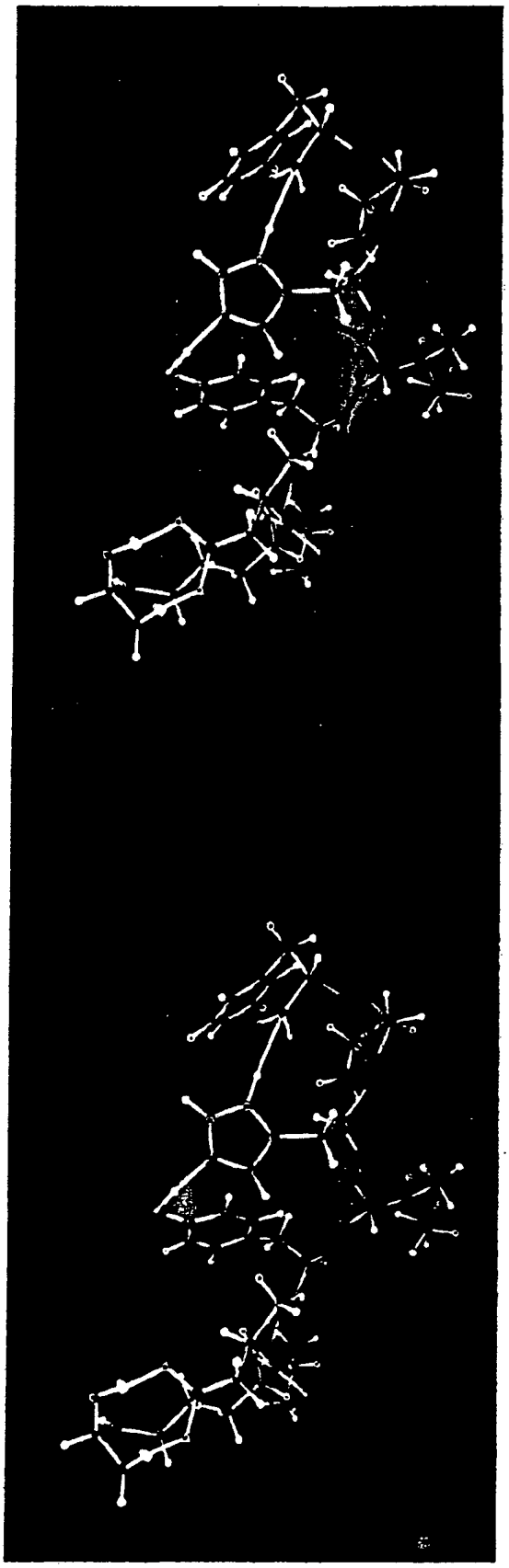


FIG._6

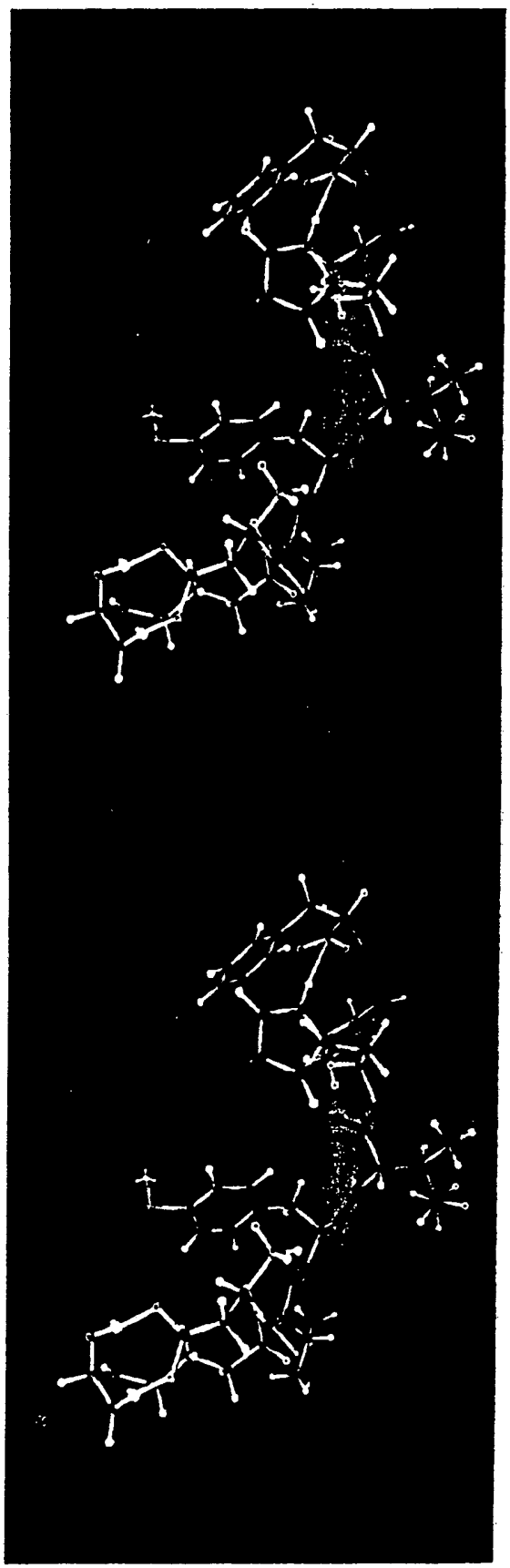


FIG._7A

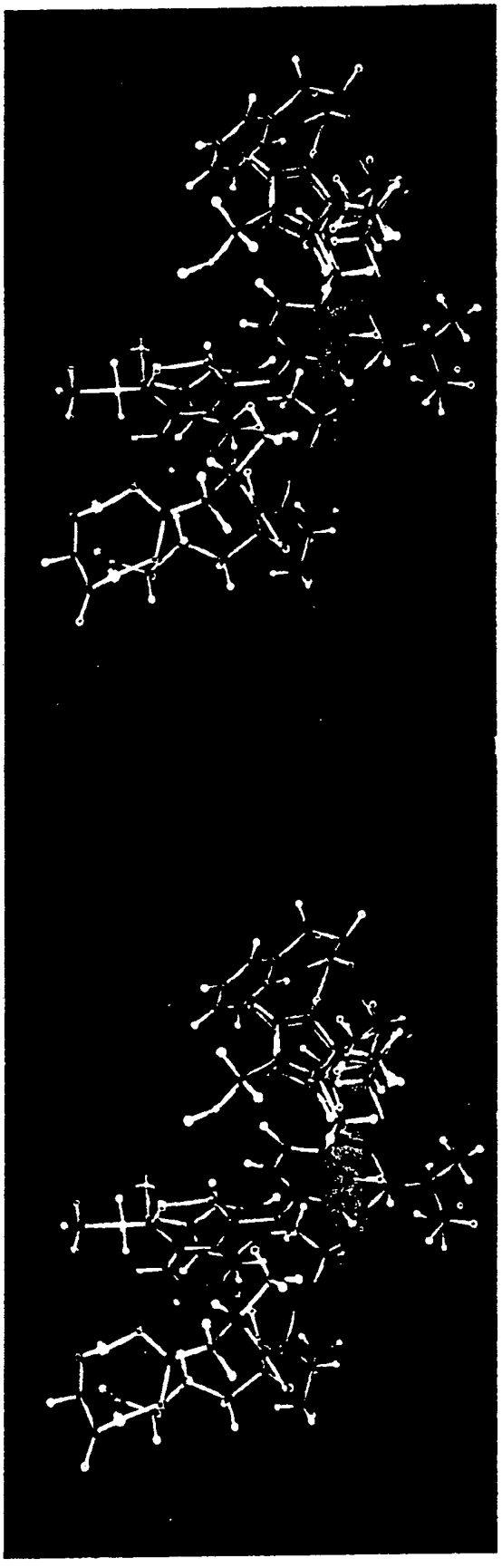


FIG._7B

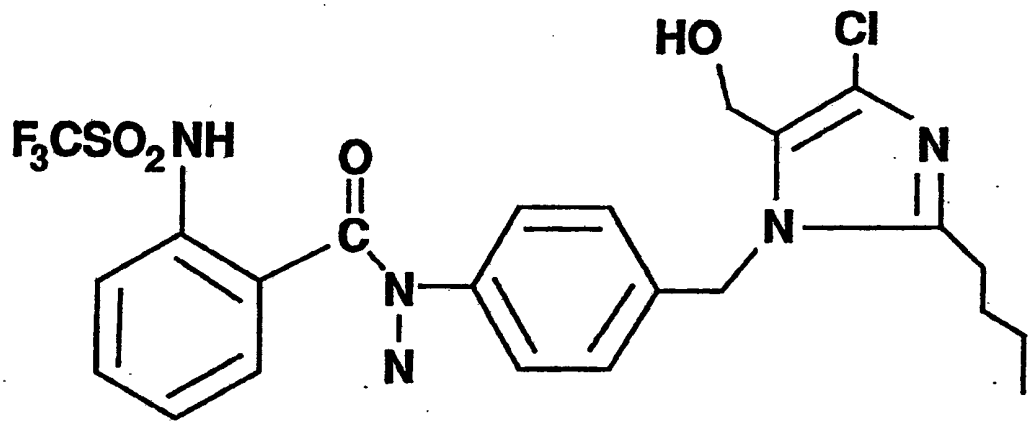


FIG._7C